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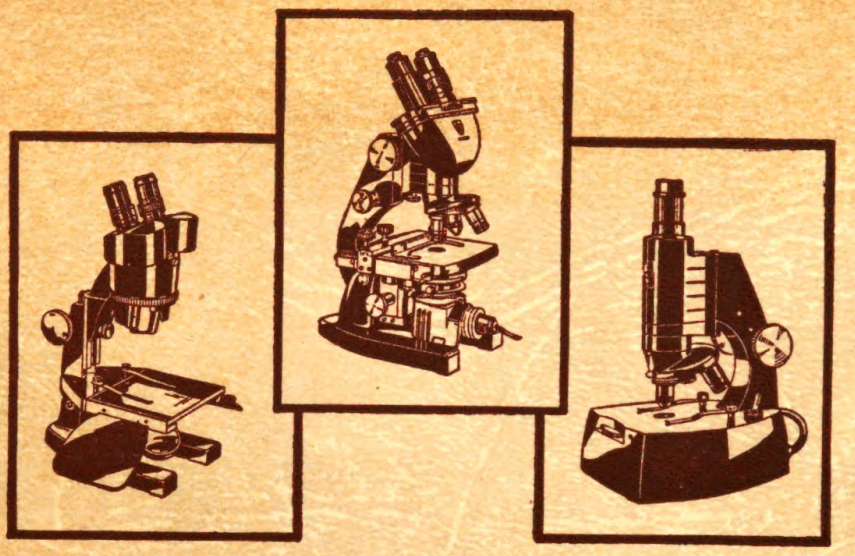

The Effective Use

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The Effective Use

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by

Oscar W. Richards, *Ph. D.*

Research Supervisor for Biology

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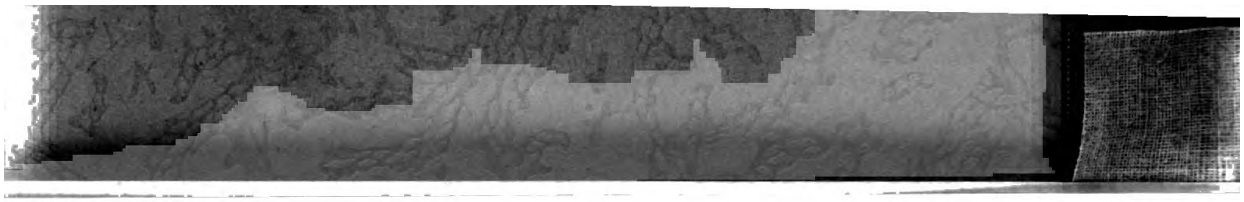

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Basic Summary of Use

I. Microscope *without substage condenser.*

A. Place specimen on stage over the center of the opening in the stage. By sliding it under the stage clips it will be held in place.

B. Place lowest power objective (one with shortest mount) a short distance above the specimen.

C. Turn concave side of mirror toward light and move until field of view is as uniformly lighted as possible.

D. Raise body tube by turning coarse adjustment knob until the specimen is seen clearly. Readjust mirror, if necessary, to obtain best illumination.

E. For greater magnification turn nosepiece to bring higher power objective over the specimen.

II. Microscope *with substage condenser.*

A. Using lamp *which has no lenses to concentrate its light.* (Illuminated surface type)

1. Put specimen on stage and objective in place as in A and B above.

2. Use *plane* surface of mirror to light field of view. Raise body tube to focus specimen.

3. Remove eyepiece and open iris diaphragm of substage condenser so that back lens of the objective is nearly filled with light (90 to 100 percent). Focus condenser up and down until the objective is as uniformly filled with light as possible.

4. Replace eyepiece. If field is not lighted, moving the lamp nearer to or farther from the mirror may help.

B. Using lamp *with condensing lenses and iris diaphragm.*

1. Place lamp 8 to 10 inches in front of microscope, and turn so that the light falls on the center of the mirror.

2. Focus lamp by moving lamp bulb or lamp condenser so that the filament image is focused on the iris diaphragm of the microscope condenser. Closing the lamp iris will increase the visibility of the filament image. (The lamp should be adjusted so that the filaments and the reflected images of the filaments are in between each other as given in the instructions for the lamp used.)

3. Place specimen on stage and focus microscope on the specimen. Close lamp iris and raise or lower microscope condenser until the lamp iris is seen sharply in focus with the specimen. Open lamp iris until field of view is filled.

4. Remove eyepiece and adjust microscope iris until its edge is seen just within the back lens of the objective.

5. If the light is too intense, insert neutral filters to reduce it. Changing either diaphragm from the correct position just found, will reduce the efficiency of the microscope and decrease the illumination.

For other methods of illumination and the why of the rules one should read the manual and books on microscopy. The above rules are given to aid the beginner, but can not take the place of knowledge.

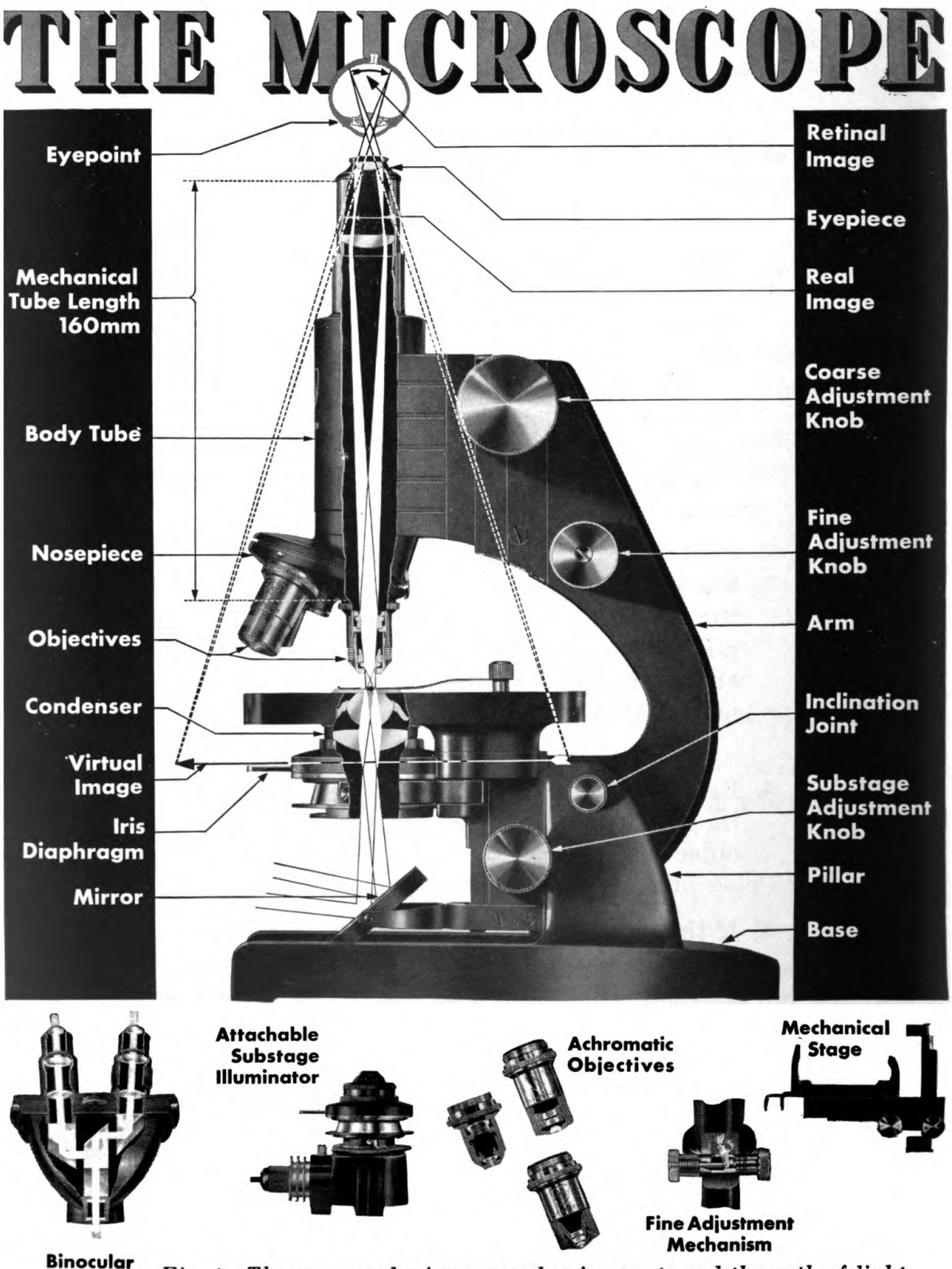


Fig. 1. The compound microscope showing parts and the path of light through the instrument.

1. Elementary Methods

A **Simple Microscope** is an ordinary magnifying glass. Many materials may be examined with the lens held in the hand. When some time is spent studying a specimen, dissecting, or mounting material, it is convenient to have the magnifying glass held on an adjustable stand. Some stands have a glass stage to support the specimen, a fixed mirror for transmitted light, and a friction-type focusing mechanism for the lenses. Others have rack and pinion focusing, with an adjustable mirror and detachable arm rests. These stands may be equipped with a corrected doublet magnifying glass or a highly corrected triple aplanat magnifier. Usually magnifications of less than twenty diameters are used.

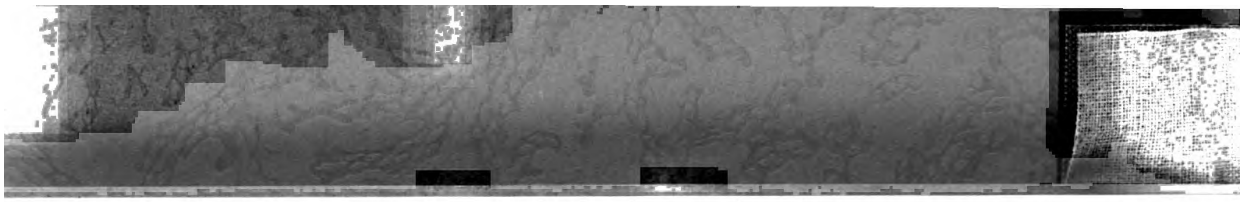
The **Compound Microscope** differs from the simple microscope in that it has two separate lens systems. The one nearest the specimen, called the objective, magnifies the specimen a definite amount. The second lens system, the eyepiece, further magnifies the image formed by the objective, so that the image seen by the eye has a magnification equal to the product of the magnifications of the two systems. This is the linear magnification or total magnification of the microscope system as a whole. The individual or initial magnification of the objectives and eyepieces is engraved on each such part.

The image formed by a compound microscope is inverted; the object is seen upside down and reversed so that the right

Fig. 2. Folding hand magnifiers.

Fig. 3. Utility magnifier.

Fig. 4 Basic compound microscope.



side is at the left. Movement is reversed also, but one soon learns which way to move the specimen slide.

The names of the various parts of the microscope are shown in Fig. 1. The basic type of compound microscope, Fig. 4, consists of the eyepiece, objective, and a tube which holds them at the proper separation. The instrument is focused by a rack and pinion, and may be mounted on any convenient support. Laboratories use them for reading scales and measuring, and in the shop they are attached to machines for observing the work done.

To achieve easy portability and convenience of manipulation, such a microscope is usually mounted on a stand with a stage to support the specimen. A mechanism is provided under the stage, such as a rotatable disc diaphragm or an iris diaphragm, for regulating the amount of light on the specimen. The light may be furnished by a built-in substage illuminator or by some external source. Fig. 5 shows a Spencer Scholar's Microscope, a simple compound type having a built-in illuminator, used in elementary and high school biology courses.

Proper use of the Scholar's Microscope in Fig. 5 is readily accomplished. The stiffness of the focusing control can be immediately adjusted to individual preference by grasping the right-hand focusing knob very firmly and turning the left-hand knob against it. To tighten, turn left-hand clockwise; to loosen, turn it counterclockwise.

Place a specimen slide under the low power objective (the short one). Turn the focusing adjustment knob to lower the body tube as far as possible. Illuminate the specimen by plugging the attached cord into any regular 110-120 volt electrical outlet, and flick the toggle switch on the right of the instrument. Now look into the microscope, and turn the focusing adjustment slowly to raise the body tube until the specimen is seen, at first fuzzy, and then finally sharp and clear. If there is too much light, so that the specimen can not be seen easily against the glare, turn the disc diaphragm under the microscope stage until a smaller opening (aper-

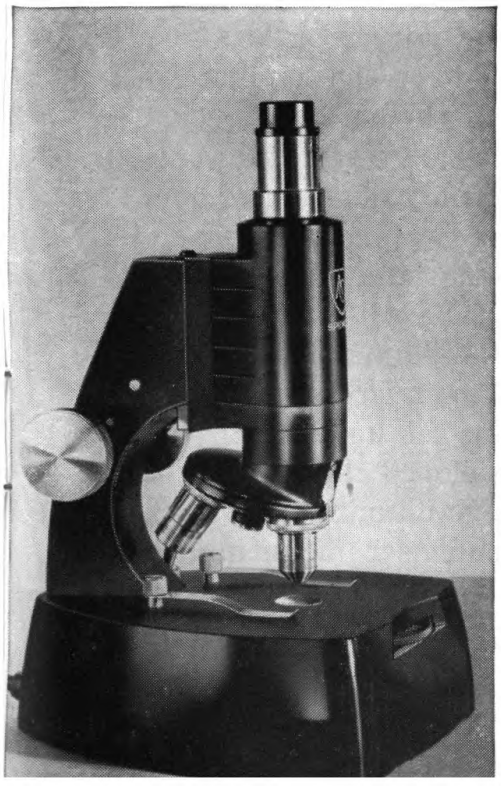
ture) is under the specimen. There are five apertures of various sizes. In general, one should use an aperture of such size that the field is fully and fairly evenly illuminated.

Once the object or specimen is in focus, it is possible to change objectives (by rotating the nosepiece) with very little refocusing because they have been made parfocal.

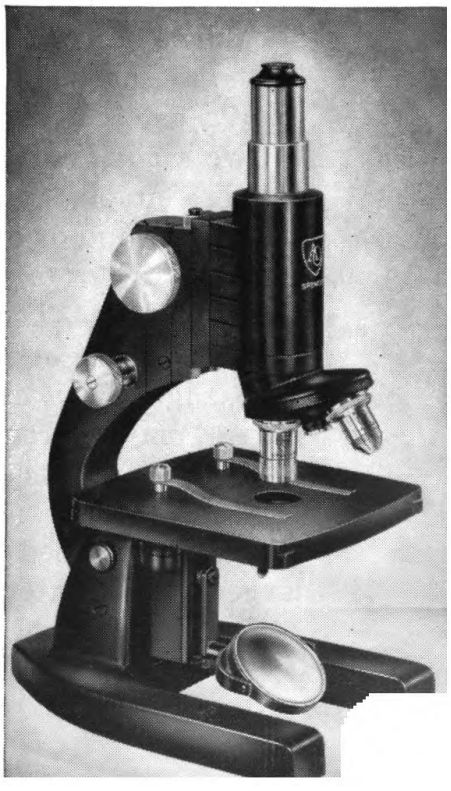
Higher powered, oil immersion objectives are not recommended for use on this type of microscope stand because they require the use of a substage condenser for proper illumination and a fine adjustment focusing mechanism.

The Laboratory Microscope illustrated in Fig. 6 is a somewhat heavier and more advanced type. It has a very slow-moving fine adjustment for precise focusing of the higher powered objectives, after the approximate focus has been found with the coarse adjustment. This fine adjustment ceases to work when the objective touches the specimen, reducing the probability of damage to the specimen or objective.

The instrument is equipped with a substage iris diaphragm which may be opened or closed slowly to any sized aperture for illuminating the object. Below the iris diaphragm is a



Left
Fig. 5.
Scholar's microscope with built-in illumination.



Right
Fig. 6.
Laboratory microscope without condenser.

mirror which is used to reflect light from an external source to the specimen.

The manner of using the Laboratory Microscope is about the same as the Scholar's Microscope, except that the illumination must be furnished by an outside source; such as, a lamp or daylight. With a specimen in place, use the low power objective, and, looking through the microscope, move the mirror until light is reflected up the tube and the field is fairly evenly lighted. After the specimen is in sharp focus, some readjustment of the mirror may improve the lighting. When the specimen appears to move as the microscope is focused, the light is not passing up the center of the microscope. Correct this by moving the mirror to a more central position.

Daylight may be used for illuminating the microscope by turning the mirror so that light from a window is reflected into the microscope. If the *concave* side of the mirror is used. the light is more intense, because the concave mirror acts like a lens to condense the light from the whole area of the mirror onto that covered by the objective. Daylight is of variable quality and not always available. Consequently, **artificial light** is generally used for microscopy. Sunlight should never be allowed to fall on the mirror or into the lenses of the microscope. It is not good for the microscope and its use is likely to injure the eye of the observer.

Two kinds of **microscope illuminators** are in common use. One is a simple lamp which has a uniformly illuminated surface of ground or opal glass. The other type has a focusable condensing lens to project an image of the lamp filament into the microscope. The use of the latter type will be discussed in Chapter 7. A small light source of the illuminated surface type, Fig. 7, may be placed in front of the microscope, or the mirror may be removed and the lamp set underneath the microscope condenser. A recently developed illuminator of the same type is the attachable substage illuminator, **Fig. 8,** which is fastened directly to the condenser mounting. A larger and brighter lamp of this type is shown in Fig 9.

The lamp should be moved towards or away from the mir-

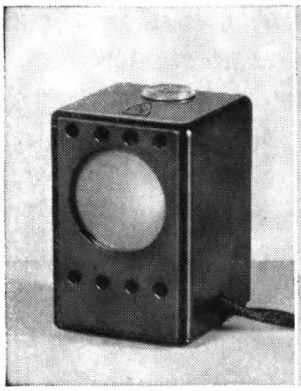


Fig. 7. Bakelite substage lamp.

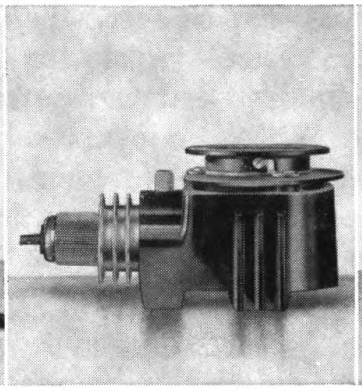


Fig. 8. Attachable substage illuminator.

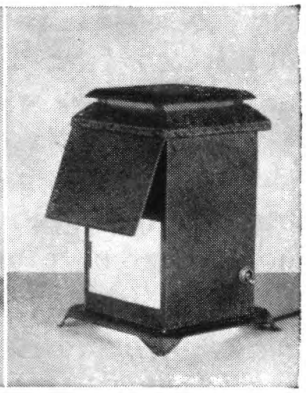


Fig. 9. Chalet-type microscope lamp.

ror until the field examined is evenly filled with light. When the concave mirror is used, this distance may easily be determined by placing a pencil on the lamp and then moving the lamp until the pencil point is in focus with the specimen. To make sure that the illumination is good, remove the eyepiece from the microscope and look at the back lens of the objective. This lens should be evenly and completely filled with light. If not so filled, the lamp and mirror should be readjusted until the best illumination is obtained.

Greater magnification is accomplished by using lenses of higher power. In order that the total size of the microscope itself need not be increased, the field seen by the higher power lenses must be smaller than that covered by the lower power eyepieces and objectives. As illustrated by Fig. 10, unless the object is carefully centered when looking at it with

Fig. 10. Incorrectly centered object not in field of higher power objective.

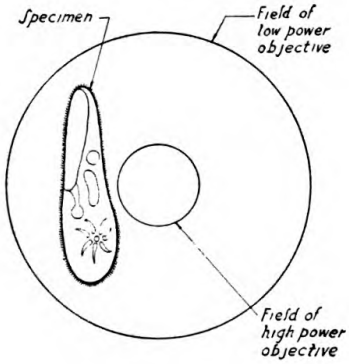
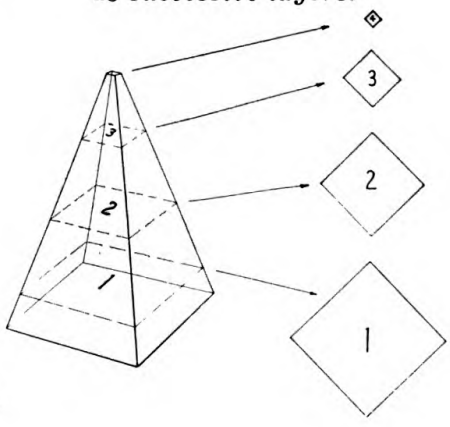


Fig. 11. Depth is seen as successive layers.



the low power, it may not be seen when a higher powered objective is turned into place by rotating the nosepiece. Modern objectives are centered and parfocalized when used on a revolving nosepiece. When the low power objective is in focus it is possible to turn the next higher power objective into position by rotating the nosepiece and see the object in nearly correct focus. A slight turn of the fine adjustment will bring the image into sharp focus.

As the microscope is focused up and down on a specimen, it will be noticed that only a thin layer of the specimen is in focus at a time. If one were looking at a semi-transparent pyramidal object, Fig. 11, the base would be seen as a large square and smaller and smaller squares will be seen as the microscope is focused upward. The greater the magnification, the thinner is the layer in focus at one time. In order to understand the nature of the specimen, it is necessary to focus up and down until the picture of depth within the specimen is built up in the mind's eye.

Some 16mm objectives are made so that the front part of the mount may be unscrewed, leaving a 32mm lens with a magnification of four diameters. Another type has the front lens system mounted on a stirrup which may be swung to one side when the lower power is desired. Thus the lenses may be used for both medium and low magnifications.

A variety of objectives and eyepieces are available and increasing magnifications within the range of the microscope may be obtained in relatively small steps by an appropriate

Fig. 12. The condenser is easily removed and replaced in a fork mounting.

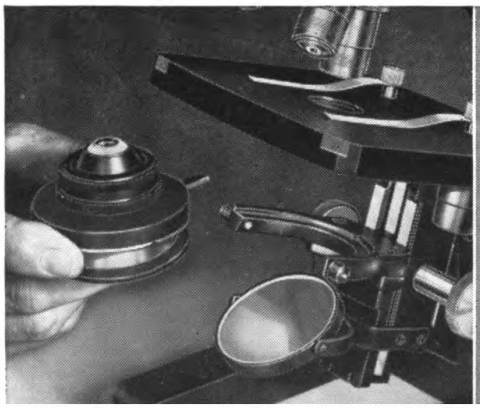
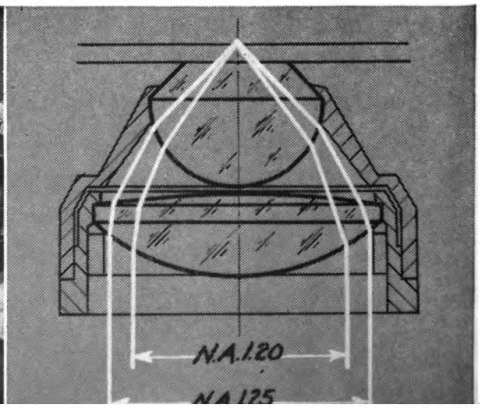


Fig. 13. Path of light through the condenser.



choice of optics. The lower the magnification used, the brighter the specimen will appear and the greater will be the depth of focus and size of the field seen. As the magnification increases, the field seen becomes smaller, the depth of focus is less, and more light is required to see the specimen. The table in Chapter 7 gives some of this data. It is preferable to use the lowest adequate magnification.

Two achromatic 4mm objectives are available with a numerical aperture of 0.66 and 0.85 respectively. The former has a long working distance necessary to focus on the Bright-Line Blood Counting Chamber. The latter cannot be used for this purpose because of its shorter working distance, but it has greater resolution and passes more light to the eye.

It is not possible to fill the back lens of a 4mm (high dry) objective adequately with light unless a **condenser,** Fig. 12, is added to the microscope. The condenser brings the light from the mirror to a focus on the specimen, as shown in the diagram, Figs. 1, 13 and it extends it from the specimen with a sufficiently wide cone to fill the back lens of the objective. The condenser has adjustments for raising and lowering it and an iris diaphragm aperture adjustment at the lower end. With daylight or a surface illumination type of lamp, the condenser is raised or lowered until the field is as evenly illuminated as possible. The iris diaphragm is opened or closed until the back lens of the objective, seen when the eyepiece is removed, is filled uniformly with light. The *plane or flat side* of the mirror should be used with the condenser.

Better vision results when it is possible to use both eyes simultaneously. A **binocular body** on the microscope, Fig. 14, permits the use of both eyes and gives some appearance of depth, although true stereoscopic vision is not obtained with this body and a single objective. The binocular bodies have either vertical or inclined eyepiece tubes for greater comfort of the user. The binocular body has two adjustments. One changes the distance between the eyepieces until both eyes see a single field. This adjustment is an interpupillary adjustment. In other words, the centers of the lenses in the

two eyepieces should be at exactly the same distance apart as the centers of the observer's eyes. The other adjustment compensates for any difference between the observer's eyes. To make this adjustment, the microscope is focused so as to be sharp to the right eye. Then the right eye is closed and the adjustment on the left eyepiece turned until the image is equally sharp for the left eye. Then both eyes should see the same image equally well.

The amount of light going to each eye is less than the amount passed by the monocular body tube, because an equal portion of the light is deflected to each eye. In addition there is a loss of light due to absorption and reflection in the prisms of the binocular body. With dense specimens more intense illumination may be necessary. (See Chapter 7.) The binocular bodies are interchangeable with monocular bodies. By loosening the screw at the bottom of the arm just back of the nosepiece, the body may be removed. Tightening this screw locks the bodies into proper alignment with the optical system. Earlier models have this locking screw at the top of the arm.

The **oil immersion objective** found on the more complete microscopes requires more careful use than the others. In order to show the detail of which it is capable, it has to be illuminated by a wider cone of light. In fact the illumination required is beyond the angle which can be obtained unless the spaces between the objective and the cover glass and between the slide and the condenser are filled by a suitable immersion oil of nearly the same refractive index and dispersion as the glass of the slide and lenses. Crown and cedarwood (not leaf) oil are satisfactory. (*Cf.* p. 20) Before turning the oil immersion lens into position, place a small drop of oil on the slide in the center of the field, or as some operators prefer, on the lens. Then bring the lens into place slowly so as to prevent the formation of air bubbles in the oil. The condenser diaphragm will have to be opened in order to fill the back lens of the objective adequately with light. The full amount of detail which may be resolved with this objective is not obtained unless there is also an oil contact between

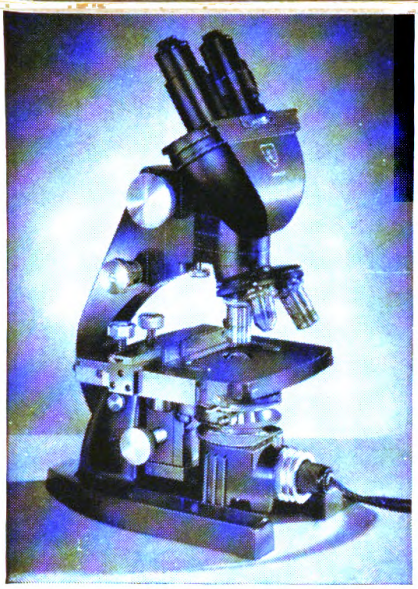


Fig. 14. Binocular monobjective microscope.

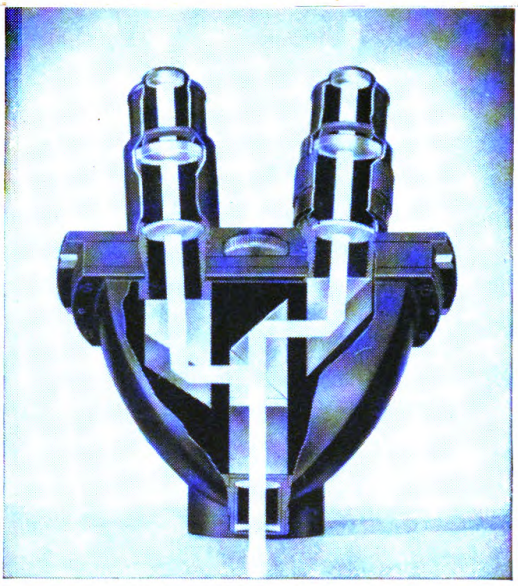


Fig. 15. Path of light through the binocular body.

the condenser and the under side of the slide. Efficient use requires that a drop of oil be placed on the condenser when it is racked down so that when the condenser is brought back into place the oil fills the space between the condenser and the slide. (See Fig. 38 a & b in Chapter 7). When full resolution is not required, the condenser may be used dry.

The oil immersion objective focuses very close to the specimen. Unless No. 1 cover glasses, 0.13-0.17mm thick, are used, it may not be possible to bring the lens close enough to focus on the specimen. Considerable care is necessary not to damage the lens by rough handling. After use the immersion oil must be wiped off with lens paper.

The fine adjustment is graduated and may be used to measure the vertical distance. Focus on the bottom and then on the top of the specimen, and read the difference on the scale. When the measurement is not made in air the scale value must be multiplied by the refractive index of the material measured. The indices of refraction of some common mounting materials are: balsam and damar 1.52-1.54, cedar oil 1.52, Clarite 1.54, glycerine 1.46, Hyrax 1.73±, Styrax 1.58±, water 1.33. It is desirable to turn the fine adjustment in only one direction during a single measurement. Avoid measuring at the extremes of the fine adjustment.

2. Preparation of Materials

Few materials may be examined with the microscope as they occur in nature. To be seen under the microscope the specimen must be thin, or sufficiently transparent to transmit enough light for observation. Only the surface of specimens not sufficiently transparent or translucent can be examined. The methods for the preparation of materials vary, so that it is not possible to give more than the most general directions. In the bibliography at the end of this booklet will be found the key references which give the special technic and further references to literature on the subject. The following general methods may be useful:

Dry preparations have limited possibilities for microscopic examination. They are usually examined with low magnification using light placed above the stage. For this type of work the stereoscopic microscope is particularly useful. (See Chapter 3.) Materials like diatoms may be mounted dry on a slide under a cover glass to take advantage of the difference in refractive index between them and air. Small microorganisms, e.g. bacteria, may be smeared on a slide, allowed to dry and examined with or without staining. Metal surfaces and minerals, after proper polishing, are usually examined dry.

Many **temporary preparations** reveal more detail after they are mounted in a drop of water and covered by a thin cover glass. The cover glass is necessary to prevent the liquid from getting onto the surface of the objective and also to give a flat surface so that the light enters the objective, as shown in Fig. 16. The rapid evaporation of water is annoying and other fluids, like glycerine, when they will not injure the specimen, may be used instead of water. While examining water mounts, it is wise to add a drop of water to the edge of the cover glass from time to time to replace that lost by evaporation. As the water evaporates the cover glass is drawn down toward the slide and may crush or injure the specimen.

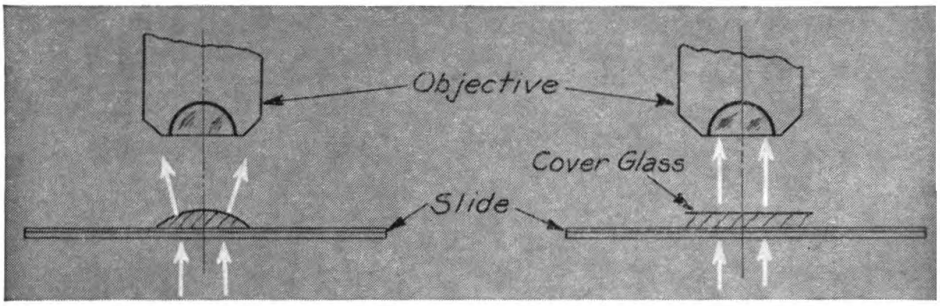
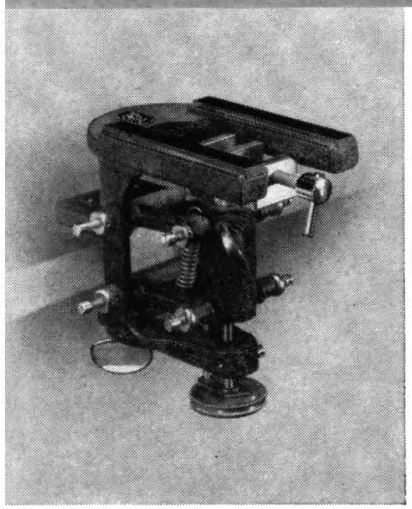


Fig. 16. A cover glass is used to flatten preparation.

Permanent preparations are made by mounting the specimen in a transparent material underneath a cover glass. Dry specimens may be mounted as they are found. Any moisture in a specimen must be removed before mounting in media like Balsam, Clarite or Damar by dehydrating the preparation with alcohol, dioxan, or some other suitable chemical. Balsam solutions may be slightly acid and therefore may fade the dyes in time. Synthetic resins are replacing these media to a considerable extent. When dehydrating the specimen would damage it unduly, it may be mounted in glycerine jelly.

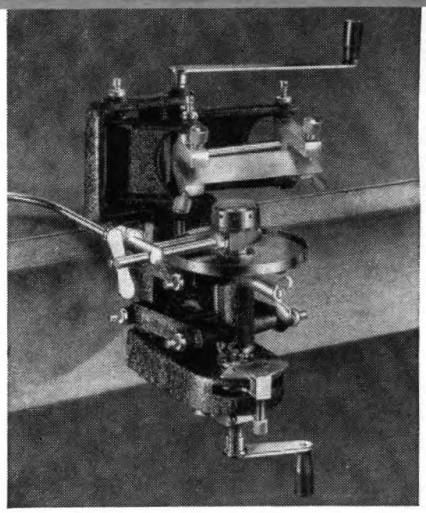
Specimens for examination may be *selected* from a mass of material by means of a forceps or, if in a liquid preparation, by means of a capillary pipette, made by drawing out in a hot flame the tip of a medicine dropper. If a bit of plant or animal tissue is placed in a drop of water it may be *teased* apart by means of two dissecting needles until some of the parts are separate and small enough for examination with the microscope. Harder materials are prepared by *crushing* and then sifting out the parts desired. Another form of separation is to place the material in a bag of cloth and to knead it under water so that the part not desired will separate out and may be washed away.

Some materials are separated by *filtering*. *Sedimentation* is another common method for separation of materials. A mixture is suspended in water or other liquid and allowed to settle. The coarser particles settle out rapidly and the finer



Left
Fig. 17. A table microtome.

Right
Fig. 18. Clinical microtome with freezing attachment.



particles more slowly. By taking a sample at varying heights within the column or at various times, it is frequently possible to get suitable sized specimens for examination. Such methods are often used with abrasives or with soils.

Selective solubility with various chemicals is a convenient method of removing the material not desired. For instance, cloth may be treated with alkali to dissolve the wool and leave the cotton fibre so that the proportion of the two materials may be estimated. *Chemical methods* have been devised, and it is possible to make chemical analyses under the microscope. In fact, this procedure has many advantages. Very small amounts of both the unknown and the reagent are required, and because small amounts of solvent are used, the reaction may be observed in concentrated solutions. The reactions are often clearer than macro procedures with more dilute solutions.

Fig. 19. Precision rotary microtome.

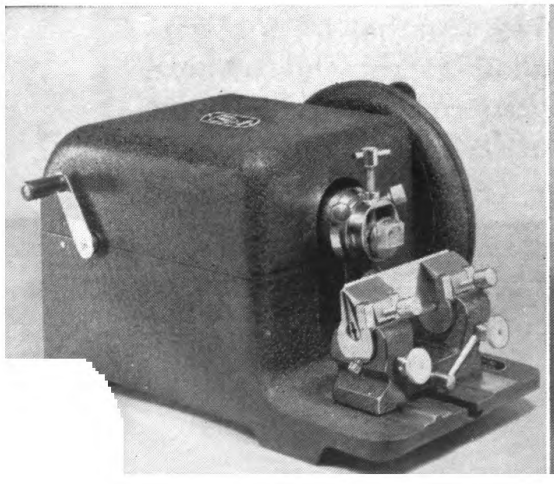
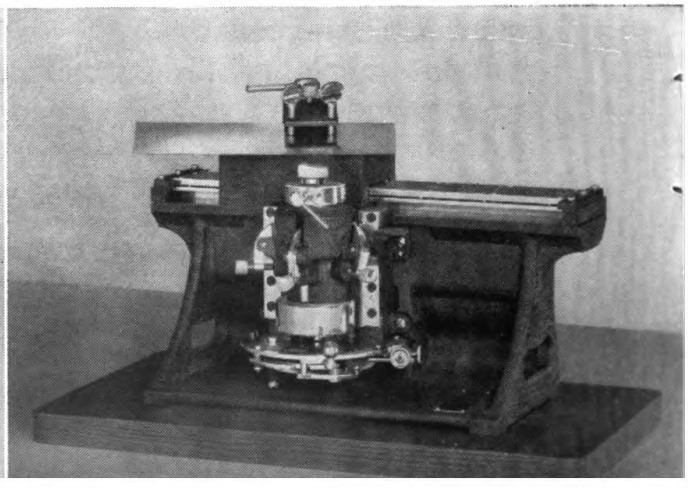


Fig. 20. Precision sliding microtome.



Oils or solvents are used as mounting media to *clear* the material not desired. For instance, in studying adulteration, mounting in paraffin or clove oil may make starch transparent so that crystals or other materials present may be seen.

Other useful methods include differential swelling, stepwise dehydration, incomplete salting out, and isothermal distillation.

Many specimens have to be *sectioned* before they are thin enough for examination under the microscope. Hard materials, like rocks and metals, have to be sectioned with a diamond saw or other type of cutting machine, ground down to thinness and polished after they have been mounted on the slide. Metal surfaces may require chemical etching to reveal the detail.

Less solid materials may be *frozen* on a freezing microtome, Fig. 18. The specimen is mounted on the cutting chamber and frozen with carbon dioxide gas. Other materials are first dehydrated and then thoroughly saturated with paraffin or celloidin, and, after this has hardened they are cut on microtomes. A **rotary microtome,** Fig. 19, is used for cutting paraffin preparations because the successive sections form a ribbon. Fig. 20 shows a **sliding microtome** which has the greatest general utility as it may be used for delicate specimens imbedded in celloidin or for cutting large and difficult specimens.

Skillful preparation of materials is of fundamental importance. If a specimen is badly prepared the most expert microscopist may not be able to identify it or make out its structure. Learning the technic of preparing specimens, like learning all technics, usually requires the making of all the mistakes once. It is a field in which skill increases with practice, and the beginner should not feel discouraged if his first efforts are not entirely successful. While it is possible to learn technic from the study of books, it is an easier and shorter process to learn from watching another skilled person and from study under supervision.

Immersion Oil

An immersion medium in microscopy provides an optically homogeneous path between the cover glass and the front lens of the objective. The early recognition of suitable optical properties in cedarwood oil has made it a traditional standard for immersion media. Consequently modern immersion lenses have been designed for use with this medium or one of closely similar optical properties.

In addition to a suitable refractive index and dispersion, a satisfactory immersion oil must possess the following physical properties: It should be chemically inert. It should be free from a tendency to spread or creep. It should remain fluid and not harden rapidly when exposed to air. Its optical properties should be stable and not change with age.

Immersion fluids recommended for use with Spencer homogeneous oil immersion objectives are: Cedarwood Oil prepared by us for use with Spencer objectives. Crown Oil available from us and most scientific supply houses.

Other fluids should not be used for Spencer immersion objectives unless they possess the characteristics mentioned above.

Paraffin oils have low refractive indices and are not recommended.

Methyl Benzoate should never be used because its low dispersion destroys the color correction of the oil immersion objectives.

3. The Stereoscopic Microscope

The **Stereoscopic Microscope** is fundamentally two microscopes, one for each eye, combined for convenient use in a single instrument. Instead of single objectives, the nosepiece carries paired objectives mounted in a single housing. Each pair may be readily changed for different magnifications. The two eyes view the same specimen from separate angles, and the result is real perception of depth (Fig. 21). The Spencer objectives view the specimen at an included angle of 12°, and the eyepieces converge at an angle of 8°. A specimen is seen right side up through the stereoscopic microscope and movements are normal and not inverted as with the monobjective microscope. The erect image facilitates examination of material which must be moved about, as well as the dissection of organisms under the microscope.

The paired objectives of this microscope make stereoscopic vision possible. On the other hand, with paired objectives the numerical aperture is limited to about 0.12 and, as explained in Chapter 7, there is no advantage in using very high magnification. The Spencer Stereoscopic Microscope can provide magnifications from 6.3 to 144 diameters. The instrument is balanced to give brilliant lighting, good resolution, and a flat field with considerable depth of focus. As the magnification is increased, the size of the field and depth of focus decrease, and more light is required to see the specimen. It is preferable to use the lowest magnification adequate for the material to be examined. Detailed information is given in Catalog M86, available on request.

The microscope may be equipped with a single pair of objectives, but usually a rotating nosepiece carrying two or three pairs of objectives is used, Fig. 22. An inclination joint allows tilting of the microscope. For the examination of solid material, the upper part may be removed from the base by loosening a thumb screw. The glass stage may be removed and the microscope placed directly over material in Syracuse or other small dishes. A metal plate with one side white, the

other side black, may be placed just under the glass stage to provide a contrasting background. A mechanical stage may be attached to the stage for the control of specimens mounted on slides or in counting chambers.

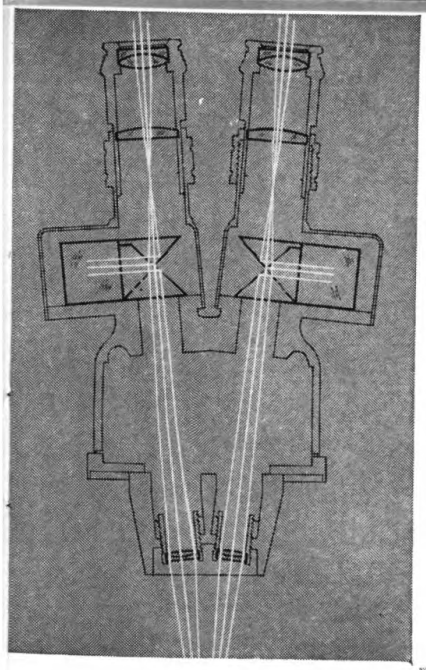
Some stereoscopic microscopes are mounted on a pivot over a board for the examination of medium sized specimens. For the greatest ranges required, some microscopes are mounted on adjustable arms with separate rack and pinion adjustments, as shown in Fig. 23. This mounting can be swung around to examine from different angles large specimens on the table and in aquaria.

Transparent or *semi-transparent materials* are examined much the same way as with the compound microscope. Light is reflected to them by means of a mirror. The concave mirror will concentrate more daylight, or light from a broad source, onto the specimen. With concentrated sources the plane mirror is useful. If in doubt, try both.

The prism housings which support the eyepieces are rotatable and should be moved in or out until the distance between the center of the lenses is the same as it is between the center of the observer's eyes. This is easily done by looking through the microscope. When they are exactly at the right distance one sees the depth of the specimen. When the two eyes of the observer are different the left-hand eyepiece tube may be raised or lowered by the knurled ring until the focus is the same for both eyes.

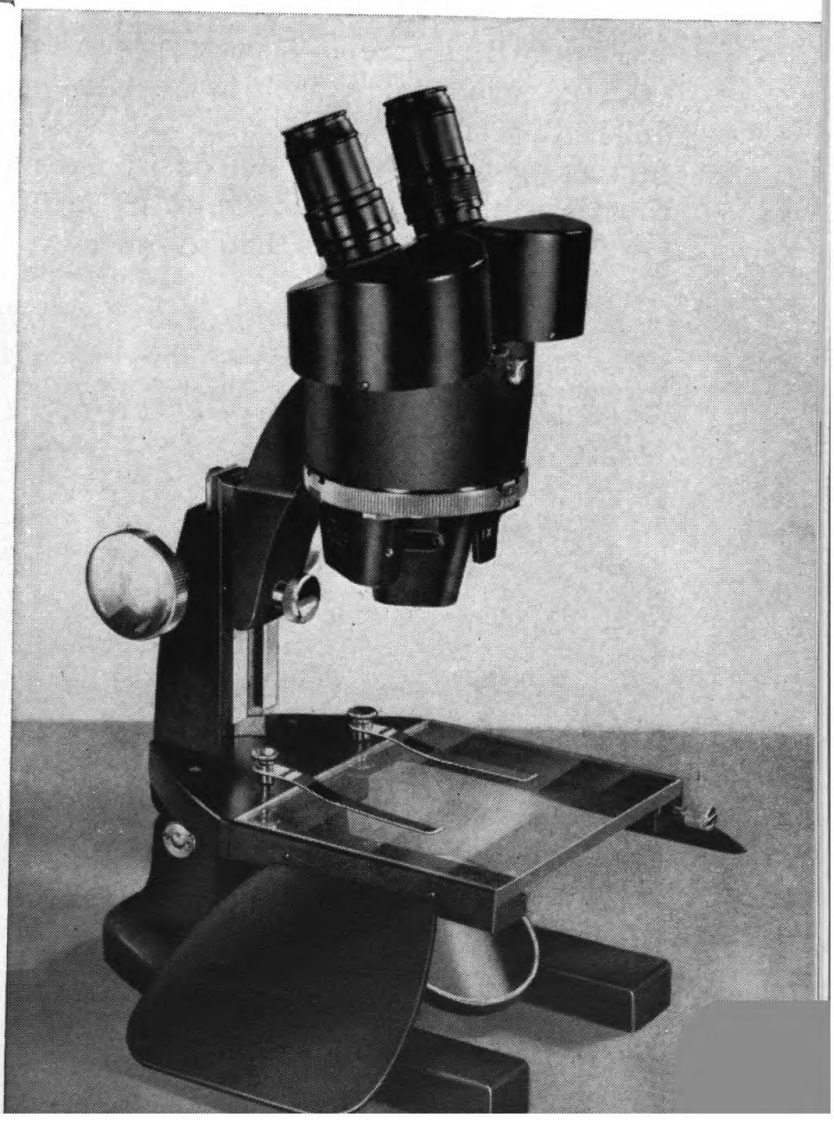
For the examination of *opaque materials* it is desirable to have a lamp, designed for these instruments. By screwing the lens mount in or out, it is possible to concentrate the light within the field examined by the various objectives of the microscope. When the microscope is moved from specimen to specimen, it is convenient to fasten the lamp onto the microscope, for which set screws are provided.

Only very general rules can be given for illumination; the best method must be found by trial. Sometimes the light should strike the specimen at an acute angle, the lamp being set as near the objective as possible. For the examination of



Left

Fig. 21. Path of light through stereoscopic microscope.



Right

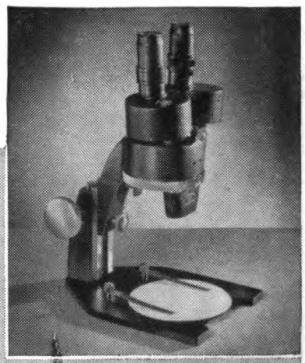
Fig. 22. Stereoscopic microscope with removable base.

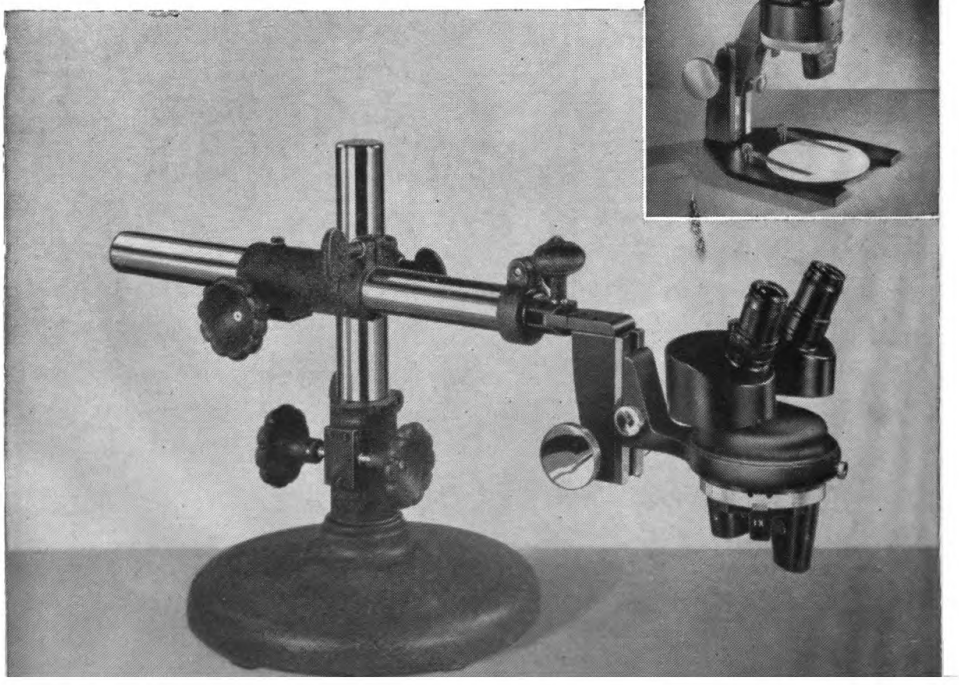
an indented surface it may be desirable to set the lamp for parallel light by screwing the mount of the lens in nearly as far as it will go and setting the lamp so the light just barely grazes the surface of the specimen. For transmitted light, the lamp may be set in front of the microscope and focused on the specimen by means of the mirror. Some specimens show best when the mirror is in the center and the light thrown directly into the microscope. Others require setting the mirror to one side so that the specimen is lighted obliquely to reveal detail. With seeds and other small objects, lighted from above, there is often enough light passing between them that may be reflected back by the mirror, so that they can be seen to advantage on a partially lighted background.

Spencer Stereoscopic Microscopes are used in industry to examine small parts, such as bearings, surface quality of finished parts, and to detect flaws in castings. Ceramics and textiles are examined, as well as ore and minerals. Seeds and contaminants in food and drugs are identified. Applications are found in agriculture, botany, zoology, and medicine.

Below
Fig. 23. Stereoscopic microscope on adjustable arm.

Right
Fig. 24. Stereoscopic microscope for opaque objects.





4. The Care of the Eyes

With proper care the microscope may be used for hours at a time without undue fatigue. To be comfortable, have the microscope on a table at such a position that you may look into it without stretching or cramping the body. Use both eyes with the binocular body or alternate the eyes when using a monocular body tube. The eye not used should be kept open to avoid tiring the muscles by trying to hold it tightly shut. At first you will be bothered by seeing with the eye not looking through the microscope, but a very little practice will permit you to keep both eyes open and see with one. Holding the hand in front of the other eye and gradually taking it away will help one become accustomed to working correctly.

It is important that the amount of light be adjusted so that there is enough to see the object without strain and yet not have unnecessary light to cause fatigue from glare. Continual use of the fine adjustment will afford the greatest comfort in observation.

For best vision with binocular bodies the distance between the eyepieces should be adjusted carefully until both eyes see the same field. Different eyepieces have the eyepoint at different levels; which may require readjustment of the interpupillary distance when eyepieces are changed.

Spectacles and Eyeglasses

Spectacles and eyeglasses used to correct near or farsightedness need not be worn when using the microscope. The microscope is merely focused differently with the fine adjustment. However, if the spectacles have a correction for astigmatism it is desirable that they be worn to avoid headaches.

When the camera lucida or reticules are used for drawing, counting or measuring, it is usually necessary to wear spectacles, if these are normally worn for reading.

High eyepoint oculars are available and their use is desirable when spectacles are worn.

5. The Care of the Microscope

Successful microscopy requires skill and the proper care of the instrument. The microscope is a precision instrument made from valuable materials by expert workmen. With reasonable care it will last a long time, but a single bit of carelessness may ruin it.

The microscope should be carried by its arm and, when not in use, should be placed in its case or properly covered to protect it from dust. When the microscope is brought from a cold to a warm room it should be allowed to warm up gradually before being used.

The lenses must be kept meticulously clean. Dust should be loosened and brushed off with a camel's hair brush and the lens cleaned with lens paper. Optical glass is generally softer than window glass and is easily scratched by ordinary cloth or when dust particles are not removed before polishing. Special lens paper is available and it is poor economy not to use it.

Dust on the eyepiece lenses is seen as specks which rotate when the eyepiece is turned while looking through it. Dirt on the objective prevents clear vision and the object looks as if it were in a fog. If a wet preparation touches the objective lens, the lens will have to be cleaned before one can see clearly through it. An eyepiece should always be kept in the tube to prevent dust from collecting on the back lens of the objective. If it does collect there, clean it off carefully with a camel's hair brush or blow it off with an aspirator. An all rubber ear or infants' enema syringe is useful, and is obtainable at most drug stores.

Should dust settle on the prisms or on the glass protection plates of the binocular body, blow it off with air from an aspirator bulb. Blowing the breath on lenses will cover them with minute drops of saliva which are removed from the lens with difficulty. Compressed air from laboratory pipes may contain traces of moisture or of oil from the compressor, and should not be used unless an absorbent cotton filter is placed on the discharge tube.

If the field does not appear clear, it is well to examine the lower surface of the objective with a magnifying glass. Any dirt or damage to the lens may then be seen easily.

Objective lenses are carefully adjusted at the factory, and should not be taken apart except where they have been made to separate (*e.g.* the divisible 16mm objective and the older style immersion objective with funnel stop). The definition depends on all of the component lenses being centered and the right distance from each other. If they must be taken apart, it should be done at the factory where facilities are available for testing the reassembly.

The dry objectives, the condenser, and the eyepieces may be cleaned with distilled water when a liquid is necessary; an immersion objective and condenser top lens with xylene. Only the smallest amount of solvent should be used and the lens should be wiped dry with fresh lens paper immediately after cleaning. Should the immersion oil become gummed on the lens it should be cleaned off with the least amount of xylene and then the excess wiped off promptly with lens paper. Do not soak the lens with xylene or other solvent because the mounting of the lenses may be damaged if it gets beyond the seal of the front lens into the objective.

In tropical regions mold will grow on dirty lens surfaces when the relative humidity and temperature exceed 80°F. When the optical surfaces are kept clean, mold growth can be avoided, or minimized, by keeping the optics in a dessicator, or the microscope in a warmed cabinet.* Other suggestions include the use of radium or fungicides.†

The surface of the microscope is finished with black enamel or metal plating and requires little more care than keeping it clean and free from dirt. These finishes resist most lab-

* Hutchinson, W. G. *Sci. Mon.*, 1946, 63:165-177.

† Turner, J. S., McLennan, E. I., Rogers, J. S., & E. Matthaei. *Nature* (London). 1946, 158:469-472.
Vicklund, R. E. *Ind. & Eng. Chem.* 1943, 38:774-779.

oratory chemicals and ordinarily a little water is all that is necessary for cleaning. The stage of the low power stereoscopic microscope is removable and also the plate with the dark and white sides. Both may be washed in neutral soap and water.

The slides of the rack and pinion should be cleaned occasionally with a small amount of oil or light grease. The fine adjustment does not require oiling. After a time wear may make the coarse adjustment turn too easily to support the body tube. To increase the tension grasp the control knob of the coarse adjustment with the right-hand and turn the left-hand knob clockwise, Fig. 25. To reduce the friction turn the left-hand knob counterclockwise. With former models the paired screws at the top of the stand may be tightened slightly to give sufficient friction to hold the body tube properly.

Fig. 25. Adjusting tension of coarse adjustment knobs.

Careless handling or dropping may disturb the adjustment of the optical parts of the microscope. If the instrument does not seem to perform properly and there is no dirt on the objective or the eyepiece, it may mean that some of the prisms have become shifted. Do not attempt to adjust any of the prism systems but rather send the instrument to the factory where tools and tests are available for adjustment and for making certain that the adjustment has been done properly.

If the stereoscopic microscope fails to give a fused image or causes eyestrain, the objectives may have become decentered; this may be tested readily by focusing onto a ruler or some other object with a straight line. Move the line to the extreme left side of the right eye's field. Then notice whether or not the line is in the corresponding position in the field for the left eye. Test the lens also by moving the line to the top or bottom of the field. If the line is not in corresponding positions in the two fields the objective should be sent to the factory for proper adjustment. The lenses should be adjusted only by a person who has had factory training.

Testing microscope lenses is a difficult task, and one which should only be attempted by a skilled microscopist. Instructions for the use of test plates and test objects are given by Beck (1938), Belling (1930), and Spitta (1920). Adequate comparison requires the best illumination and adjustment of the microscope and considerable experience with lenses of different quality so that definition and aberrations may be evaluated. (Cf. also Chapter 7.)

6. Accessories for the Microscope

The **mechanical stage** is one of the most useful accessories. It is a mechanism for moving the specimen by rack and pinion or screw movement slowly in either of two mutually perpendicular directions. Mechanical stages may be obtained built into the research microscope, or added on and easily removable from the laboratory microscope; or they may be bought separately and added to most square microscope stages (Fig. 26). The mechanical stage is particularly helpful when it is necessary to search a specimen to make certain that no part has been missed; for instance, examining a stained sputum smear for tuberculosis bacteria. The mechanical stage is helpful also when counting or measuring. The position of a field to which reference is to be made later, may be marked on the label of the slide by the position of the indices and verniers on a graduated mechanical stage.

Petrographers and critical microscopists who require their objectives precisely centered with respect to the optical axis of the microscope, prefer the **quick-change nosepiece,** Fig. 27, to the rotating nosepiece. The quick-change nosepiece has individual centering screws so that each objective may be centered and once centered may be quickly taken off and replaced to exactly the same position.

When two people are using the same microscope, or where it is desired that two people see the same view at the same time the **demonstration eyepiece,** Fig. 28 is useful. Spencer

Fig. 26. Graduated mechanical stage.

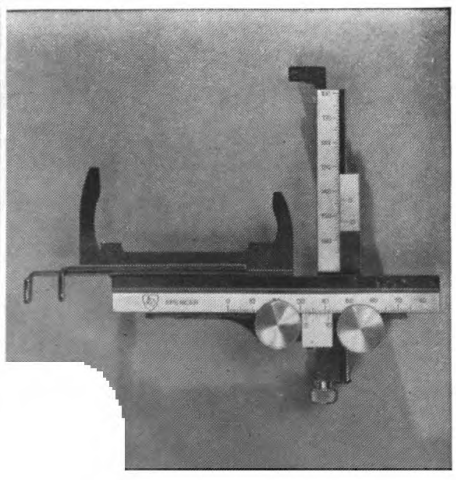
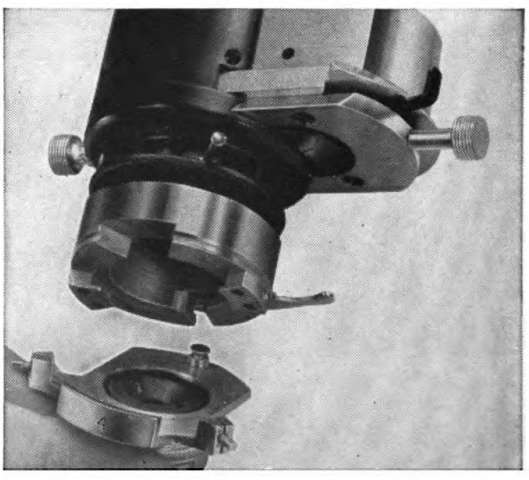


Fig. 27. Quick-change nosepiece.



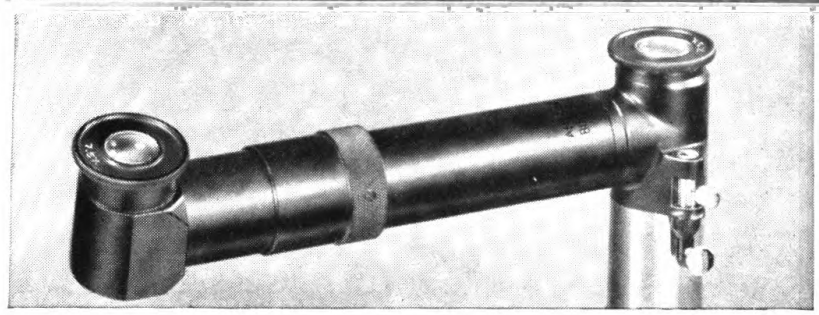


Fig. 28. Demonstration eyepiece.

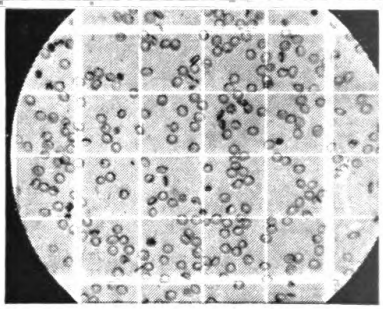


Fig. 29. Blood cells on Bright-Line counting chamber.

Demonstration Eyepieces may be obtained with a built-in pointer so that one person can point out a definite region of the specimen examined and both may be certain that they are looking at the same part of the specimen. The demonstration eyepiece is particularly useful in teaching.

Several kinds of equipment are available for **counting.** Probably the best known is the haemacytometer. The *Spencer Bright-Line Haemacytometer* is filled with diluted blood, and the number of blood cells in the different squares of the ruling, Fig. 29, are counted under the microscope. From these counts the total number of blood cells may be estimated. The counting chamber is made so that the under side of the cover glass is 0.1mm above the ruled surface to control the volume over the ruled area. The Bright-Line counting chamber is also used for yeast, dust, and other counts. The lines are easily and distinctly seen because they are ruled in a metal surface fused in glass.

Another type of counting equipment has a chamber of a definite depth, but instead of having an extensive ruling on the large area, a *ruled reticule* is placed in the eyepiece, Fig. 30a. As one looks into the microscope the ruling is seen in focus with the slide and since the volume is known, the number of organisms or particles may be estimated from the count. This method is used for counting protozoa in water and for dust counting. The Sedgwick-Rafter and Howard Mold Count apparatus illustrate this equipment.

A definite volume of fluid (milk, for instance) may be spread over a known area on a slide. Then a crosshair eyepiece, Fig. 30b, having a diaphragm or etched circle of the proper diameter to give the correct area of view, is placed into the microscope. After the film of milk is stained, the

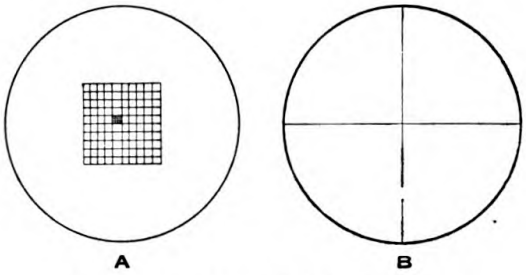


Fig. 30-a. b. Ruled reticules for eyepieces.

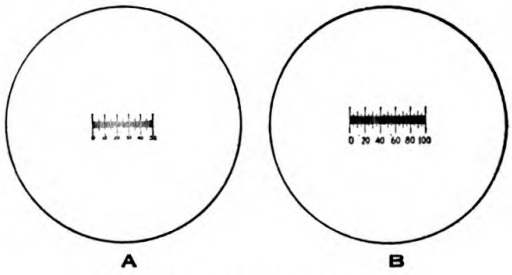


Fig. 31-a. b. Measuring reticules for eyepieces.

number of bacteria in the field are counted. The estimate from the count is accurate only when the field of view covers the correct area. This must be adjusted at the factory, so that the diaphragm aperture covers exactly the proper length on a *stage micrometer*. Counting equipment for milk is made in accordance with standards set by the American Public Health Association.

Measuring equipment for the microscope consists of a scale or reticule placed in the eyepiece and calibrated against a standard stage micrometer. Variously ruled scales, Fig. 31, may be used in any eyepiece. The top lens of the eyepiece is unscrewed and the reticule is placed with the ruled side down so that it rests on the diaphragm within the eyepiece. When a considerable amount of measuring is done, it is better to have an eyepiece made with a reticule mounted in place. The better ones have an adjustable eye lens so that the scale marks may be focused separately for eyes with or without spectacles.

Measuring reticules are placed in the right eyepiece of a stereoscopic microscope by unscrewing the diaphragm from the bottom of the eyepiece. A spanner wrench for turning the diaphragm is provided for each reticule suitable for use in stereoscopic microscope eyepieces. Place the reticule in the back of the diaphragm, engraved side downward, and fasten it in place with the small circular spring which is furnished with each reticule. The diaphragm with reticule is placed back in the eyepiece and turned in until the reticule becomes clearly focused.

A third type is the **screw micrometer,** Fig. 32, which has a scale moved by a control at the side of the eyepiece. Thus,

it is possible to shift the scale which simplifies measurement, and to measure with the outside scale and vernier rather than counting the divisions seen.

All of the scales placed in the eyepieces have arbitrary length, and the apparent length depends on the magnification. Consequently, each scale has to be calibrated for use with each combination of objective and eyepiece. To calibrate, focus on a **stage micrometer** and move it until one of the graduations corresponds exactly with one of the divisions of the eyepiece micrometer, Fig. 33. The true distance *(x)* seen on the stage micrometer, which corresponds to the number of divisions *(y)* of the eyepiece micrometer, is then read and dividing this true distance by the number of divisions of the eyepiece micrometer, we find the distance each one subtends. $(c=x/y)$. The number of divisions covered by the specimen multiplied by the calibration constant *(c)* gives the length of the specimen. Once an eyepiece micrometer has been calibrated it need not be recalibrated when used with the same eyepiece, the same objective and the same tube length. If the tube length of the microscope with adjustable draw tube is changed, these values change proportionally, and this may bring the values of the eyepiece scale to an even value. A slight movement of the draw tube causes little loss of definition, but any change in tube length from the correct value of 160mm increases the spherical aberration (and reduces the definition). If small details need not be resolved a certain amount of distinctness in the image may be sacrificed for convenience in calibrating the eyepiece scale. Other accessories will be discussed in Chapter 8.

Fig. 32. Screw micrometer eyepiece.

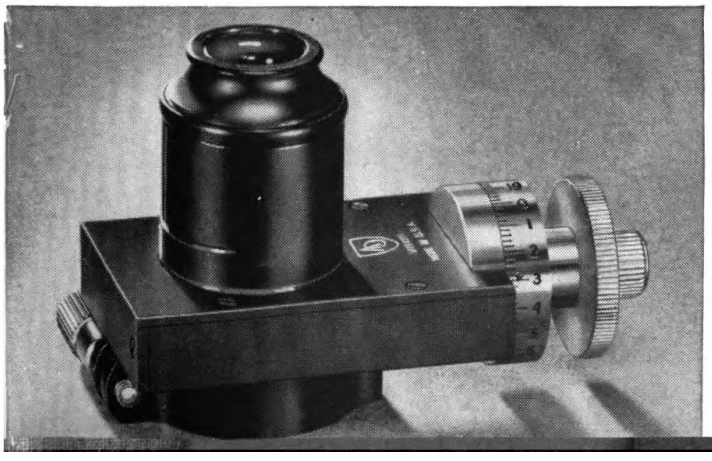
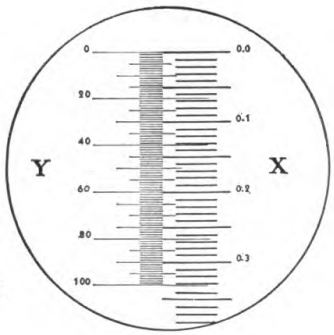


Fig. 33. Calibration of eyepiece reticule.



7. Advanced Technic

The critical use of a research microscope requires considerable skill, technic, and an understanding of the capabilities and limitations of microscopes. Some of the essentials of this work are included in this and the following chapters.

The microscope is an instrument of limited capability. The limitations arise in the nature of light used to see the specimen and the nature of the transparent materials available for lens manufacture. A further limitation on visual observation is the limited sensitivity of the human eye *(Cf.* Chapter 8). Because of the wave nature of light and the fact that the different portions of light waves interfere with each other, the image of a bright point of light is a disc and not a point. The bright central disc is surrounded by alternate dark and bright rings forming a diffraction pattern. A lens well corrected for *spherical aberration* concentrates most of the light into the central disc, loses little in the rings and gives a crisp image with good definition.

Light of different colors will focus at different points along the optical axis when a single lens is used; this is called *chromatic aberration.* To bring the colors together lenses of different kinds of glass are combined. For the highest correction crystal fluorite is used as well as glass.

Other aberrations of lenses are coma, distortion, astigmatism, and curvature of field. The residual curvature of field is not serious when the lenses are used visually because the eye is capable of accommodating itself to distance. For photography special eyepieces are necessary to give a flat undistorted field. For details of the other aberrations, texts on optics and microscopy may be consulted.

Not all aberrations can be corrected completely at the same time. Spencer optics are corrected to give the best working balance so that the microscopist obtains a clear and true image of the object examined.

Magnification alone is not the aim of the finest microscopes. A given picture may be faithfully enlarged without showing any increased detail. The enlargement is not helpful unless more detail becomes apparent. Very powerful eyepiece lenses are less useful for visual observation with the microscope. They do not show more detail, and what is seen is less bright because of the increased amplification. The higher powered eyepieces decrease the area of the field seen. That is why a second microscope is not ordinarily used to magnify further the object seen with a microscope. Magnification that does not increase the visible detail is called empty magnification. In some photomicrographic, counting, or measurement problems such magnification may be useful.

The object seen with the microscope appears to be about the same distance away as a book is held when reading average size print. By general agreement, this distance is taken as ten inches. The magnification of the microscope is the ratio of the apparent size of the object as seen through the microscope to the size of the object as it appears to the unaided eye at a distance of ten inches. When the ratio applies to the linear dimensions of the object, it is called linear magnification expressed in *units* known as *diameters*, designated by ×.

For projection or photomicrography, the image of the stage micrometer is projected onto a screen at the required distance and the magnification directly measured. The visual magnification may be measured with the aid of the camera lucida and a rule placed so that it is ten inches from the top of the eyepiece. Strictly speaking, the measurement should be made from the upper focal point of the eyepiece, but this is usually near enough to the top of the eyepiece that no serious error occurs from neglecting it. The projected image on a screen ten inches from the eyepiece is smaller than the visual magnification obtained with the camera lucida because the eyepiece is serving in a different manner.

Resolution. The resolving power of the lens is its ability to reveal fine detail, and is measured in terms of the least

distance between two lines or points at which they are seen as two rather than as a single blurred object. Resolving power is a function of the wave length of light used, the lowest refractive index between the condenser and the objective, and the greatest angle between two rays of light that can enter the front lens of the objective. The numerical aperture ($N.A.=n \sin u$) is engraved on the lenses and may be used to compute the limit of resolution. The index of refraction is n and u is the half angle of the maximum cone of light which can enter the objective lens. The eye is most sensitive to green light which has a wave length of about 0.55 microns (1 micron, μ, equals 0.001mm). The size of the smallest distance between two particles at which they can be seen as two=the wave length λ of light used, divided by the numerical aperture, when no condenser is used; or, by two times the numerical aperture when the condenser is used to fill completely the back lens of the objective with light. With the low power 16mm objective (N.A. of 0.25), the distance resolved as two points is 1.1 microns with and 2.2 microns without a condenser. The oil immersion objective with a N.A. of 1.40 resolves 0.2 microns with white light. When blue light is used, the resolving power is increased, but it is more difficult to see the specimen. A further increase may be obtained by use of ultraviolet radiation, but it is necessary to record the results photographically, because the eye is not sensitive to this radiation. The resolution for other combinations may be obtained from the nomogram, Fig. 34. The presence of objects too small to be seen directly, because of lack of resolution, may be demonstrated by dark field methods. (*Cf.* Chapter 8).

The resolving power of the microscope objective indicates which objective should be used to depict any given degree of detail. However, this does not mean that the details can be seen. Before they may be seen by the eye, they must be magnified sufficiently to be within the resolving power of the eye. Different observers' eyes are not equally sensitive, but in general the magnification should be from five hundred to one thousand times the N.A. of the objective.

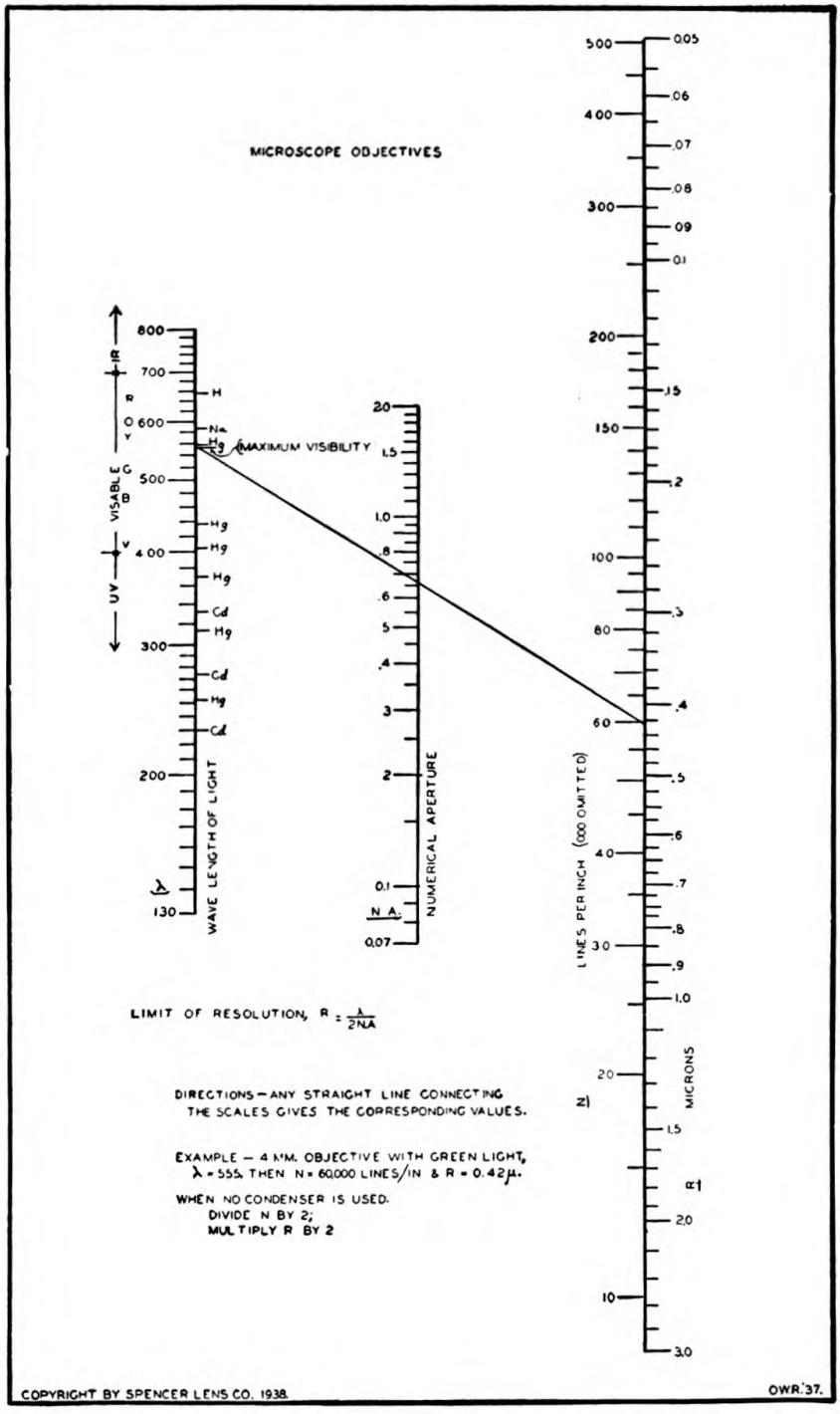


Fig. 34. Nomogram for the resolving power of microscope objectives. (Tr. Am. Micro. Soc., 1938, 57:316-318)

AO SPENCER MICROSCOPE OBJECTIVES

Achromatic Objectives

Cat. No.	*Equivalent Focus mm*	*Initial Magnification*	*Type*	*Numerical Aperture*	*Real Field† mm*	*Working Distance in mm*
101	48	2	Dry	0.08	7.0	52.5
102	40	3	Dry	0.08	5.3	35.2
104	32	4	Dry	0.10	4.0	21.0
105	30	3.5	Dry	0.09	4.2	25.4
106	24	3.4	Dry	0.08	4.3	3.7
107	25	5	Dry	0.14	2.8	16.7
109	16	10	Dry	0.25	1.5	6.8
110	16-32	10, 4.2	Dry (separable)	0.25, 0.10	1.5	6.8, 25
112	8	20	Dry	0.50	0.7	1.3
115	4	43	Dry	0.66	0.34	0.73
118	4	43	Dry	0.85	0.34	0.18
122	3	57	Dry	0.85	0.26	0.12
127	1.8	97	Oil Immersion	1.25	0.15	0.12
1292**	1.8	97	Oil Immersion	1.25	0.15	0.13

Fluorite Semi-Apochromatic Objectives

130	1.8	93	Oil Immersion	1.30	0.14	0.13

Apochromatic Objectives

150	16	10	Dry	0.30	1.29	5.2
152	8	20	Dry	0.60	0.60	0.65
154*	4	43	Dry	0.95	0.29	0.15
156*	3	56	Dry	0.95	0.20	0.11
158	2	90	Oil Immersion	1.30	0.14	0.08
159	2	90	Oil Immersion	1.40	0.14	0.05
160	1.5	120	Oil Immersion	1.30	0.11	0.07
161	3	56	Oil Immersion	1.30	0.20	0.10
1299**	2	90	Oil Immersion	1.30	0.14	0.08

* Furnished in Cover Glass Correction Collar Adjustment Mounts.

** With Built-in Iris Diaphragm.

† Achromats with 10X Huyghenian eyepiece, fluorite and apochromats with 10X compensating eyepiece.

The **working distance** of a microscope objective is the distance between the front of the objective and the top of the cover glass when it is focused on an average preparation. Numerical data for Spencer objectives are given in the table on page 38.

The **depth of field,** or the thickness of the specimen which may be seen in focus, decreases as the numerical aperture and magnification increase. The depth of focus is also decreased when radiation of shorter wave length is used. When very thin sections of the specimen are to be examined, sometimes called optical sectioning, lenses of high numerical aperture should be used. When the general arrangement of the material is to be studied, lenses of longer focal length and less magnification will generally be more satisfactory, despite their lower resolving power, because the image will be brighter and a larger field will be seen. The longer working distance of the lower power objective may also be useful.

The **amount of light** reaching the eye or the photographic plate is proportional to the intensity of the source, times the square of the numerical aperture of the objective, times the reciprocal of the square of the magnification. For example the N. A. 0.85, 4mm objective gives nearly 66 per cent more light, as well as greater resolution, than the 4mm objective of N. A. 0.66 at the same magnification, and should be used when the shorter working distance of the former is of no importance. The decrease of light according to the inverse square of the magnification is the reason for the recommendation to use the lowest magnification adequate to resolve the detail to be examined.

The research microscope, Fig. 35, is larger and more stable than other models. It has a wide choice of accessories, an improved substage, and a precise fine adjustment which is graduated to one micron and placed low for the user's convenience. It is usually equipped with more fully corrected optics.

Among the most common characteristics of lenses used for microscope objectives are the failure of light of different

Fig. 35. Research microscope with graduated circular revolving stage.

colors (different wave lengths of the spectrum) to focus at the same point (chromatic aberration) and the failure of light entering near the outer parts of the lens to focus at the same point as light entering near the center (spherical aberration).

By using different kinds of glass in combination the achromatic objectives, used on laboratory and medical microscopes, are corrected chromatically for two colors of the spectrum and spherically for one color. Further correction of aberrations in the objective requires the use of crystalline fluorite as well as glass. The fluorite or semi-apochromatic objectives are intermediate in correction between achromatic and apochromatic objectives.

Apochromatic objectives are corrected chromatically for three colors of the spectrum and spherically for two. Apochromatic objectives are necessary for the most critical microscopy and color photomicrography. They are used in essentially the same manner as are achromatic objectives. A corrected condenser of numerical aperture equal to or greater than objective aperture is required for best results. Compensating eyepieces should always be used with apochromatic objectives because they complete the correction of the optical system. If ordinary Huygenian eyepieces are used with apochromatic objectives, colored bands will be seen around the objects under observation.

In order to obtain the best image, the high magnification, dry apochromatic objectives are mounted with a correction collar for adjustment, to compensate for the thickness of the cover slip. The thickness of the cover glass affects the correction for spherical aberration and the apparent position of the specimen, Fig. 36. Spencer objectives without the correction collar are made for use with cover glasses 0.18mm thick, the thinner No. 2 cover glasses. For the sharpest image the collar is set to the known thickness of the cover glass. (It is desirable to measure the cover glass thickness at the time that the slide is mounted and to record it on the slide label.) When the thickness is not known, it

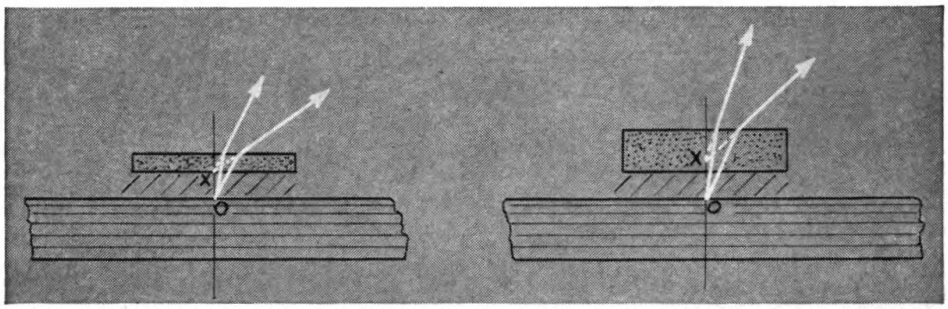


Fig. 36. The apparent position (x) of object (o) depends on cover glass thickness.

can be approximated from the performance of the objective (Belling, 1930). When no correction collar is available, the similar correction may be obtained by increasing or decreasing the length of the adjustable draw tube. The tube length is increased for thin cover glasses and decreased for thicker cover glasses. The oil immersion lenses do not need this correction because the oil makes a homogeneous path for the light to pass from the cover glass over the specimen to the objective.

With the transparent materials now available for making fine optics it is not possible in the design of apochromatic objectives to obtain perfect correction for all of the aberrations.

When spectacles or eye glasses must be worn by the operator, the high eyepoint eyepieces are more satisfactory. They permit the user to see the entire field at one time, while the ordinary eyepieces do not. The wide field eyepiece facilitates the examination of very large areas; for instance, in the study of pathological changes in sections of organs and tissues.

The simple, two-lens, Abbe type of condenser, used on the conventional laboratory and medical microscopes, is not corrected adequately for use on the research microscope. For critical microscopy corrected condensers in centering mounts are used. The three-lens, Spencer wide angle condenser has a numerical aperture of 1.40.

The achromatic and aplanatic condensers should be used with apochromatic objectives for best results. The N. A. 1.30 is a highly corrected condenser, and preferable for all work except with objectives of greater numerical aperture. In the latter case, a condenser with a Numerical Aperture 1.40 is required. These condensers are centered by closing the diaphragm until the edge of it is just seen in the back lens of the objective. Then the centering screws are moved separately or together until the iris diaphragm is concentric with the opening of the back lens of the objective. When the finest detail of which the objective is capable of showing must be examined, it is necessary that the condenser be carefully centered to the objective used and that immersion oil be placed between the condenser and the microscope slide. Unless the condenser is immersed, the air film reduces the N. A. of the objective to less than 1.0, Fig. 37. For example, with white light the resolution of an objective of 1.40 N. A. is reduced from 0.2 to 0.3μ. (Compare Figs. 38 a, b.)

The full capabilities of a microscope are not obtained unless the illuminant is efficient. Daylight is not very satisfactory because of its variability. Most critical microscopic work is done with artificial light, and for research work it is necessary to have a lamp with a condensing lens and a diaphragm, Fig. 39.

The lamp may be used in either of two ways. **Critical illumination** was generally used in former times when a white cloud or an oil lamp flame served as a light source. The image of the lamp flame was focused so that it appeared in the field of the specimen. Light from the lamp to the microscope condenser is focused so that the source appears in focus with the specimen, Fig. 40.

The disadvantages of this kind of critical illumination are that the field may be unevenly lighted and it is limited to the size and shape of the source of illumination, *e. g.* the lamp filament. The limitations became serious when the oil flame was replaced by the electric coil filament lamp which would illuminate only a narrow region when focused on the speci-

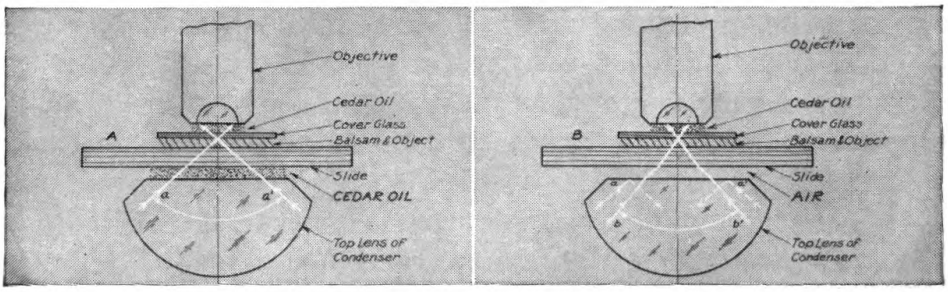
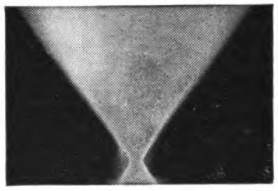


Fig. 37. Reduced illuminating cone when condenser is not in immersion contact with slide.

men. Köhler devised a type of critical illumination, which bears his name, to overcome this limitation.

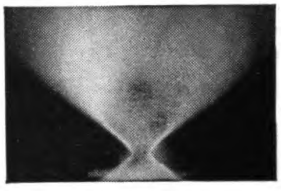
To obtain **Köhler illumination,** Fig. 41, focus the condenser of the lamp so that the filament image is in sharp focus on the diaphragm leaves of the microscope condenser. The diaphragm of the condenser may be seen by looking at the microscope mirror or with an auxiliary mirror held in the hand. Focus the microscope on a specimen and then focus the microscope condenser until the diaphragm on the lamp is in focus with the specimen. Examine the mirror to make sure that the light from the lamp falls on the center of the mirror. The iris diaphragm on the lamp (sometimes called a field stop) is now closed until no more than the field to be examined is illuminated. Now remove the eyepiece and examine

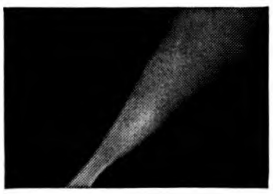
Fig. 38. Comparative cones of light.



a. N.A. 1.30 with no immersion oil on condenser top.

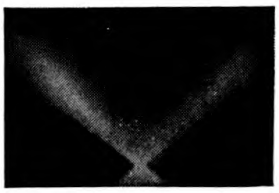
b. N.A. 1.30 with immersion oil on condenser top.





c. Oblique light

d. Dark field illumination.



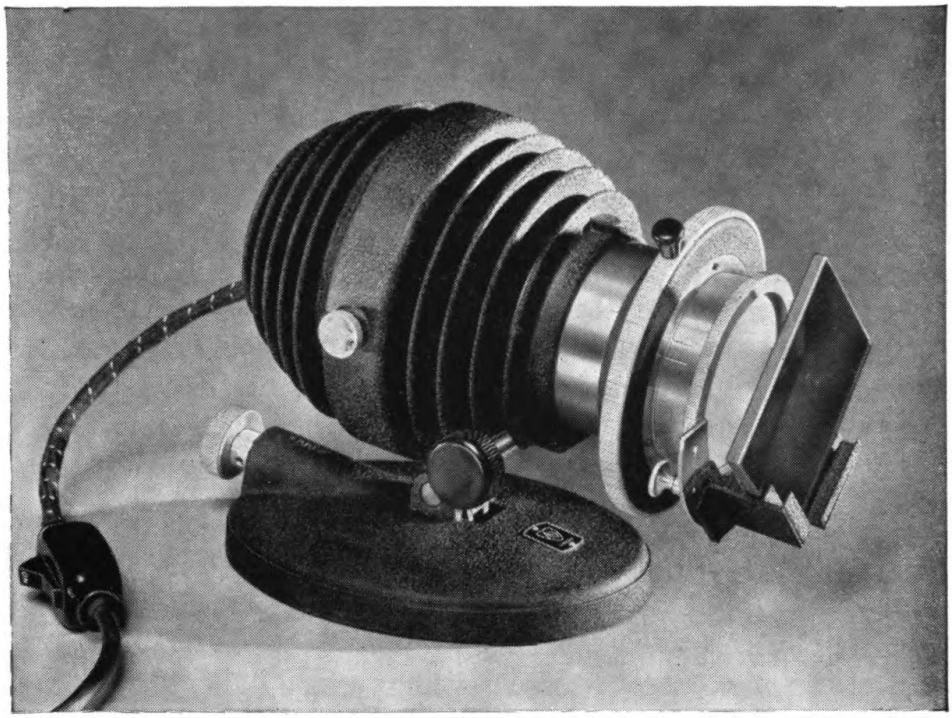


Fig. 39. Focusing research lamp.

the back lens of the objective. The diaphragm of the condenser is an aperture stop and should be opened or closed until the back lens is filled with light. An image of the lamp filament will be observed and this image should be large enough to fill the back lens of the objective. If it is not, move the lamp away from the mirror until the best balance is attained. Unless both diaphragms are thus adjusted, excess light will produce glare, spoiling definition, both visually and photographically.

The illumination is now correct, but the amount of light passing through the microscope may be too intense for comfortable observation. This light must not be decreased by closing the diaphragm of the microscope condenser because that would reduce the cone of light so that it would not fill the back lens of the objective with light. The numerical aperture used, and hence the resolving power, would be reduced under these conditions. Full resolving power is obtained only

when the back lens of the objective is uniformly fiilled with light.

The light may be reduced without decreasing the ability of the lenses to resolve detail by means of neutral density filters placed between the lamp and the microscope. By using one or more filters it is possible to adjust the light so that the object may be seen clearly without fatigue from unnecessary glare. The light from a tungsten filament lamp is yellow and this is usually made whiter by absorbing some of the excess red by means of a blue glass, or better, with a Corning Daylite Glass. The latter adjusts the color temperature of the lamp to very nearly that of daylight, provided that the correct lamp, of proper voltage, is used and lighted by electricity at that voltage.

When properly adjusted the lamp should fill the field and the aperture of the lens used, and the intensity should be adjusted for clearest vision without glare. The highly corrected condensers do not fill the field of the very low power objectives. To fill the field it is necessary to remove the top lens from the condenser, by unscrewing the top lens mount.

Unless the back lens is uniformly filled with light the diffraction patterns will not be correct and fine details will be distorted. The interpretation of form and of diffraction patterns becomes increasingly difficult and important as the limit of resolution is approached (Beck, 1938). The microscopist will find detailed discussions of the problems of resolution in the books cited in the bibliography.

Fig. 40. Critical illumination.

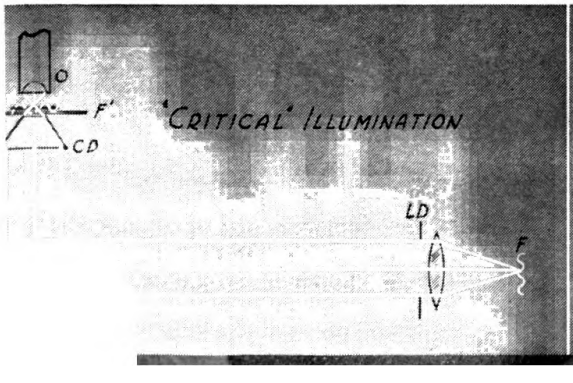
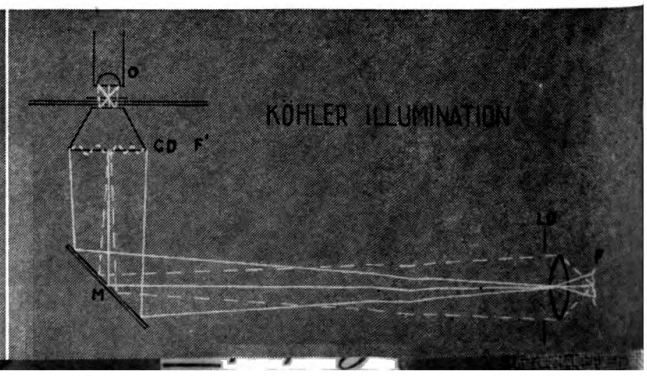


Fig. 41. Köhler illumination.



8. Visualization and Special Methods

An object is seen when the light from it reaches the retina of the eye and the resulting photochemical action initiates nerve impulses which are interpreted by the brain. The human eye can see only with radiations from 380mμ to 740mμ, and is most sensitive to yellow green light of about 555mμ. Shorter radiations (ultraviolet) or longer radiations (infrared) cannot be seen by the normal human eye and photographic recording is necessary. The vision and color sensitivity of different people varies from normal, to deficient, to complete color blindness. It is important that the microscopist know what limitations, if any, his vision has, and he should understand enough color theory to be able to use color filters effectively in microscopy.

If there are two transparent regions of different refractive index in the object examined under the microscope, the light passing through the region of contact will be bent and the shape of the material is interpreted from the bent light or *refraction image*. By this means the refractive index may be determined. The material is immersed successively in fluids of different known index until the boundary between them disappears. When the specimen is of different index there is usually a bright line surrounding the specimen. If this bright line, the "Becke line," moves in toward the specimen as the microscope is raised the specimen is of higher refractive index than the surrounding fluid.

When the specimen contains planes which reflect light it may be seen in part by the *reflection images*. Transparent materials like tissue cultures are seen as a combination of these two types of images and with the Köhler method of illumination very little detail may be visible. Contrast and, as a result, some detail may appear as the diaphragm of the microscope condenser is closed and the resolving power is reduced accordingly. Such detail may be seen after staining, or by phase microscopy.

When the different parts of the image have different

colors then the specimen is seen from its *color images*. This is the usual method of making material visible and many dyes are used in microscopy. Color filters between the lamp and the microscope may be used to increase the contrast and detail seen in colored specimens. A filter of complementary color increases contrast, and a filter of nearly the same color as the object reveals detail.

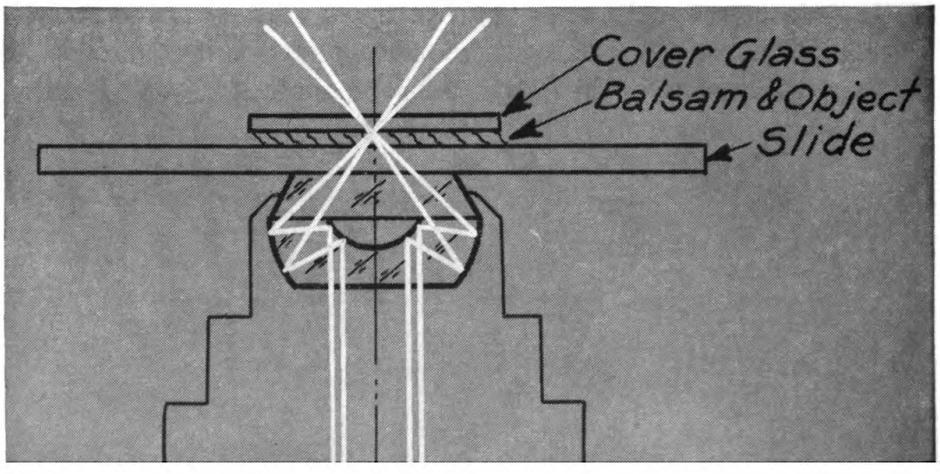
Portions of coherent waves of light interfere with each other, making brighter and darker regions called diffraction patterns, and the image seen is a result of these patterns.

Materials which fluoresce or are affected by polarized light, as described below, also give specific color images which are helpful in visualization.

The image of the object examined with the microscope formed on the retina of the eye is usually a combination of the several different kinds of images, rather than a single one. A working knowledge of the kinds of images assists in the comprehension of what is seen and lessens the likelihood of misinterpretation. Great care is necessary when the object viewed is close to the border line of smallness which may actually be seen with the microscope.

Oblique Light. If the mirror of the microscope is swung to one side so that the light passes through only one side of the condenser the specimen is illuminated by oblique light, Fig. 38c. The oblique light attachment is available as a part of the substage of the research microscope. Turning the control knob changes the obliqueness of the light. The oblique

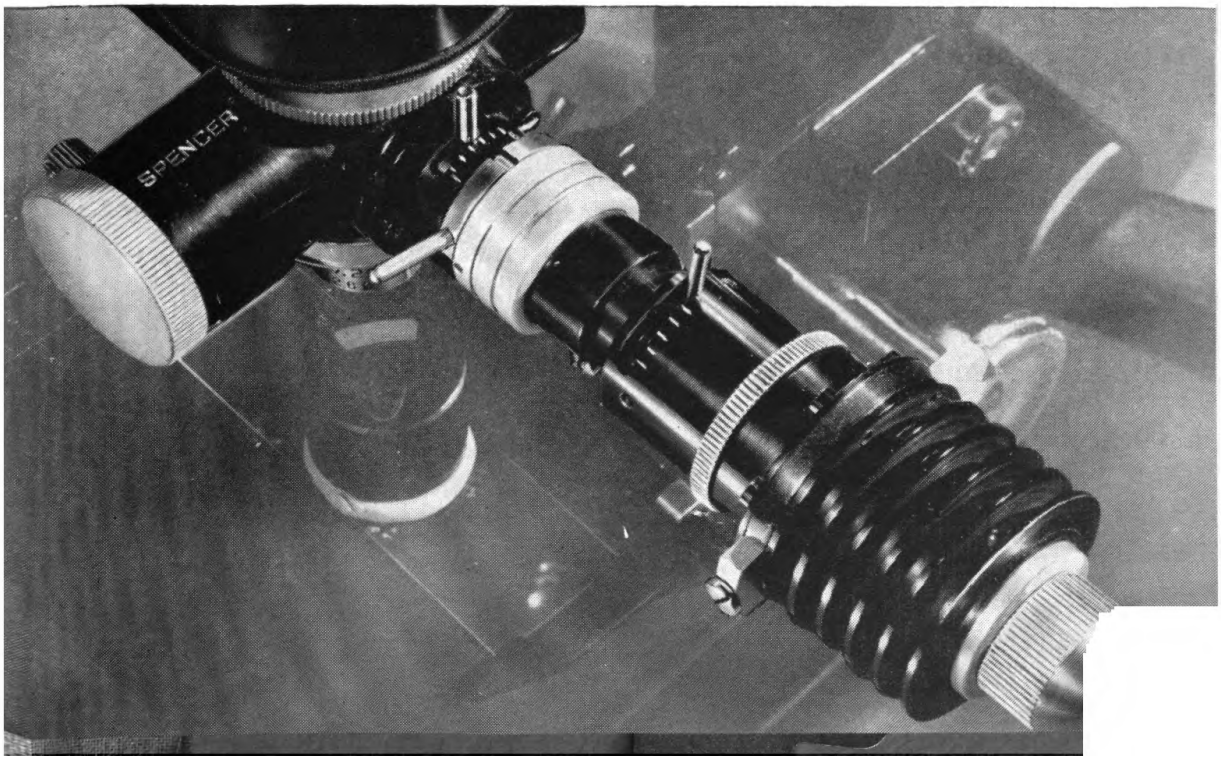
Fig. 42. Path of light through a dark field condenser.



beam of light can be moved through an arc of 90° by a corresponding movement of the control handle. Oblique light emphasizes any regular pattern in the specimen. If the light is passed at right angles to direction of greatest contrast the pattern largely disappears and great care is necessary in interpreting the images seen. Oblique light is used primarily in the study of diatoms.

Dark Field. The dark field condenser, Fig. 42, has a central dark stop which prevents any direct rays from entering the objective, Fig. 38d. The cone of light is focused onto the specimen, which appears as if it were a self-luminous body on a nearly black background. The effect is the same as the well-known appearance of dust in a beam of sunlight. Small bright objects are more readily seen against a dark field than similar small dark objects on a bright background. The dark field method reveals objects smaller than may be seen with bright field, including the presence of particles too small to be resolved. When the particles are of size below the limit of resolution of the objective used, very little can be inferred as to their shape, although the number present and approximate

Fig. 43. Vertical illuminator.



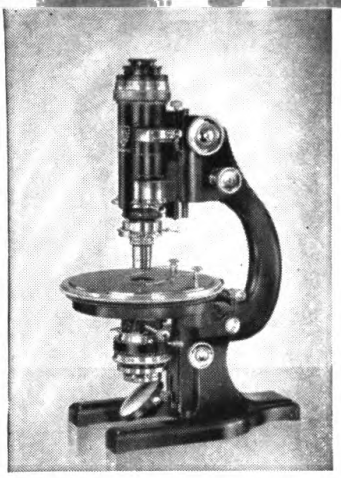
size may be estimated. The more intense the illumination, the smaller are the particles which may be discovered.

A dark field condenser or one with built-in illumination may be substituted for the bright field condenser. Dark field microscopes with hinged substage and permanently aligned illumination are available. In medicine an important use of darkfield illumination is in the diagnosis of syphilis. Dark field illumination is used for the examination of microorganisms and colloidal materials in both teaching and industrial laboratories.

The material to be examined must be placed on a slide of a thickness between 1.15 and 1.25mm to be in focus. The slide must be very clean. It is necessary that there be immersion oil between the top of the condenser and the slide. The Spencer bispheric dark field condensers are very accurately constructed, and for best results it is necessary that the condenser be carefully centered and focused. Detailed instructions are furnished with the condenser or may be obtained on request. It is important that an intense source of light be used with the dark field condenser without a built-in lamp. With lenses of Numerical Aperture greater than 0.85 it is necessary to reduce the N. A. to this value by inserting a funnel stop into the objective, or closing the iris diaphragm in the objective when so equipped. For dark field observation an objective with a built-in iris diaphragm is useful and provides convenient adjustment of the aperture.

The vertical illuminator, Fig. 43, is attached to the microscope after removing the nosepiece. The objective is screwed to the under side of the vertical illuminator. The simpler types of vertical illuminator use an auxiliary lamp, while the more complete ones have built-in illumination. Light from the lamp is reflected down through the objective onto the specimen and from the specimen back through the objective to the eyepiece and to the observer's eye. Vertical illumination is essential for opaque specimens and is used principally in metallurgy.

Polarizing attachments may be used on most microscopes. A polarizer is placed in the fork substage mount in place of

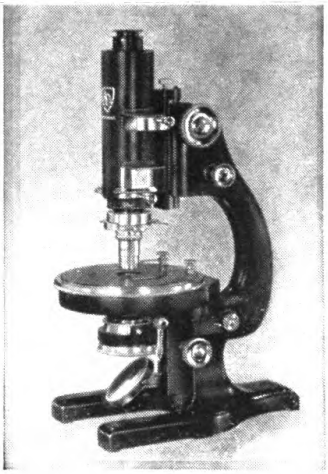


Left

Fig. 44. Research polarizing microscope (Calcite system)

Right

Fig. 45. Polarizing microscope (Polaroid system)



the regular condenser, or a Polaroid disc is placed in the condenser slot or on the lamp to polarize the light so that it vibrates in only one plane. The analyzer fits within or above the eyepiece and may be rotated until the field is dark, when the axes of the polarizer and analyzer are crossed. Anisotrophic substances placed between the crossed polarizer and analyzer show colors, and from the colors it is possible to learn a great deal about the nature of the specimen. Crystals may be identified and their optical properties measured. The images obtained with polarized light are valuable for the study of a great many materials, including chemicals, crystals, minerals, colloidal suspensions, biological fine structure, foods and drugs, and textiles. Polarizing microscopes, Figs. 44, 45, are preferable to attachments when this method is the chief use for the microscope.

Fluorescent materials emit visible light when irradiated with invisible ultraviolet radiation. Some do this naturally, while other materials must be treated with fluorescent solutions: a procedure analogous with staining. The detailed examination requires an intense source of radiation and special equipment, but simple attachments are useful for the identification of acid-fast bacteria.

Transparent specimens with detail formed by differences of refractive index or slight absorption should be examined with phase microscopy. Phase equipment consists of a special condenser, an objective with a diffraction plate appropriate to the specimen, and an aligning telescope. It may be used on the brightfield microscope.

9. Recording Observations

A permanent record of an observation made with the microscope is often desired and sometimes necessary. Some observations may be described in essay form, but in general a drawing of the object seen is the most useful record. A drawing may be facilitated by placing a reticule ruled in squares, Fig. 30a, into the eyepiece. The ruling is seen in focus with the specimen and if corresponding squares of appropriate size are drawn on the paper it is quite easy to draw the outline of the object seen from square to square.

If the microscope is tilted so that the stage is vertical and a strong lamp used, it is possible to project the image onto a paper held by a suitable easel and the image traced directly. A more convenient arrangement is to place a prism over the eyepiece of the microscope so that the image is reflected on the drawing paper. When much drawing of this type is done, special microprojectors are useful.

The **camera lucida** is used for making accurate drawings and consists of a partly silvered prism placed over the eyepiece of the microscope and a mirror for viewing the drawing surface. Some of the light passes from the microscope through the partially silvered surface to the eye, and light from the drawing surface is reflected by the mirror to the prism and to the eye. The result is that the image of the pencil and drawing is seen superimposed on the image of the

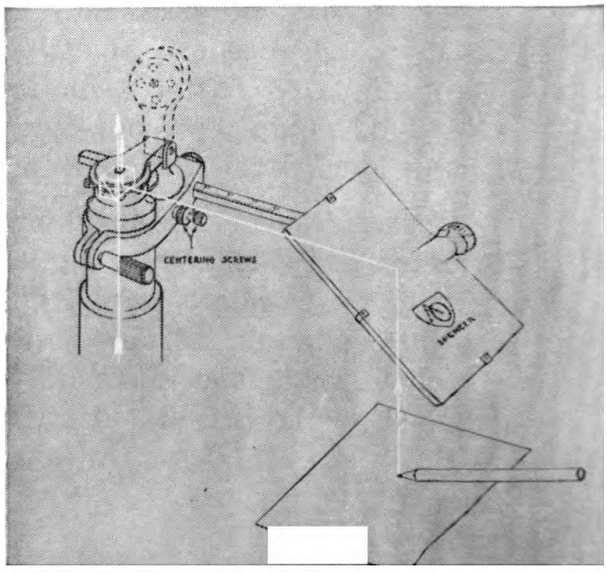


Fig. 46.

The camera lucida showing light paths.

object, Fig. 46. Underneath the prism is a rotating disc with neutral density filters. The disc is turned until the specimen is seen with proper intensity. A similar series of neutral filters is held on a collar surrounding the prism. By turning this collar until the proper one is in place between the mirror and the prism, the amount of light coming from the paper may be balanced against the light coming from the microscope. If the pencil is seen more clearly than the image, turn a denser glass into place between the mirror and prism, or use a lighter glass between the prism and the microscope to balance the light. In a bright room a screen should be used to shade the drawing from excess light.

The camera lucida may be used on monocular or binocular microscopes and on the low power stereoscopic microscope. With the monocular microscope the mirror is set 45° to the paper. With the binocular microscope with convergent eyepieces a little more than 45° is used to avoid distortion of the drawing. A special camera lucida is used with the inclined binocular body and it will be found that the image on the table is rotated 60°. To adjust the camera lucida for the stereoscopic microscope, the axis of the mirror is made parallel to the drawing surface and the mirror is tilted until there is no distortion.

To make certain that there is no distortion it is desirable to trace an object of known shape, such as a stage micrometer or the ruling of a haemacytometer. A little practice teaches how to tilt the mirror so that distortion is minimal and to balance the light so that the image and the pencil can both be seen. After making the outline drawings the prism is lifted out of view and the details are filled in from direct observation.

Reconstructions are made by drawing the image of successive sections on pieces of wax which are cut out and combined into a solid model, or the images may be drawn on separate pieces of glass or cellophane and superimposed on each other, or properly spaced in a frame. (*Cf.* Bibliography).

Photomicrography. The quickest and often the most sat-

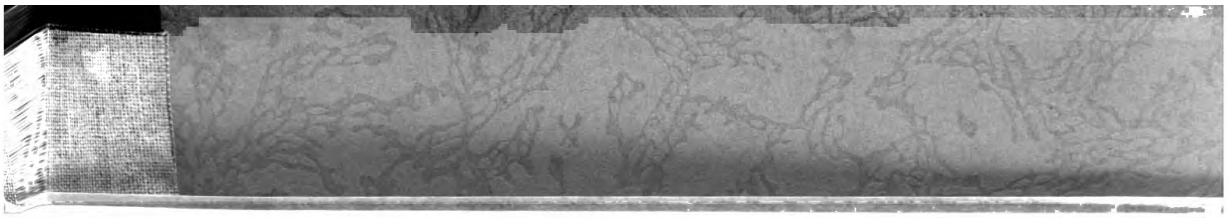
isfactory record is a photograph, especially if taken in natural color. Uncolored specimens and record photomicrographs may be made in black and white on a large variety of materials. Filters may be used to obtain the balance desired in the photograph. The photographic technic is the same as in other fields.

Photomicrographic cameras have no lenses and use only the highly corrected lenses of the microscope. Two general types of cameras are commonly used in this work. The fixed bellows camera requires little adjustment and the convenient side telescope provides rapid focusing and exposure. The fixed bellows camera is limited in magnification to the combinations of objectives and eyepieces available. The larger cameras have adjustable bellows, and the magnification may be adjusted by increasing or decreasing the bellows length to any value within the scope of the instrument.

The microscope is placed on the base of the camera, centered and focused to the ground glass or the focusing telescope. Photographic eyepieces should be used to correct the residual curvature of field of the objective and to avoid distortion. Köhler illumination is preferable. The diaphragm of the lamp (field stop) must be closed to the area of the field photographed. Otherwise glare will prevent a photograph with good definition. Apochromatic objectives, compensating eyepieces, and a corrected condenser are necessary for the best rendering of colored objects, especially if color separation negatives are made directly from the microscope.

The condenser of the microscope must be properly focused when natural color photomicrographs are taken; otherwise the background may be tinted from the chromatic aberrations of the condenser and the colors of the specimen may be affected.

The exposure may be determined by making a trial negative with a series of different exposures or an approximate exposure may be obtained from an exposure meter. Move the slide to one side so that only clear glass shows in the



field. Place a photoelectric exposure meter at the level of the plate (after removing the ground glass). The exposure is read from the meter set to the emulsion speed used, at the f/2 position on the meter. This is an arbitrary value that gives good results for the average microscope slide. Very dense or very transparent specimens may take somewhat longer or shorter exposures.

The magnification must be sufficient to make the detail resolved by the microscope large enough to be within the resolving power of the plate or film and to be seen. Enlargment of the negative, or projection of the positive must be considered, when that type of examination is to be used. Fast panchromatic emulsions resolve 40 to 60 lines per millimeter, positive emulsions around 100, and slow contrast emulsions about 175 lines per millimeter. A strict standard of definition for contact prints to be examined without further enlargement is 300 times the N. A. of the objective used. If a lower standard of definition is acceptable, the magnification may be increased to 1000 N. A. (*Cf.* Photomicrography, 1944). With ultraviolet radiation and with phase microscopy, greater magnification may be useful.

10. Conclusion

The detail given in this manual is barely enough to acquaint the user with the nature and possibilities of the microscope, some of its limitations, and brief instructions for the use and care of the instrument. The methods used in microscopy have become so varied that it is impossible to indicate more than simple methods and suggest possible applications. The importance of the microscope need not be emphasized here as it is clearly evident how the microscope has contributed to the happiness of mankind, to his information, and to the higher standards of living from improved materials and products. It is an indispensable aid to the doctor for the prevention and cure of disease. Subjects are offered to the artist and ideas for the designer.

Much time and considerable effort may be saved by study of the books and references given in the bibliography, for they offer the experience of other microscopists. Necessary skill comes with practice. Do not be disappointed if at first less is seen than anticipated. The more the microscope is used the more one learns to see, and improvement may be expected as long as the microscope is used. It is not a tool for the moment but rather one for life. Should one become tired easily with moderate periods of use, re-read the instructions and improve the methods used. Periods of several hours' work are possible with good light and a modern microscope, provided the eyes are rested occasionally.

While the microscope has the greatest practical utility in many fields, it also offers unlimited possibilities as a hobby for exploration in the world of the minute, where beauty and artistic forms predominate.

11. A Selected Bibliography

The selections given are intended to suggest current methods and to lead to detailed sources of information. Popular, non-technical, and older less available books are omitted.

These books are not sold by us, but may be obtained from your local bookstore, or the publishers. Some of the older ones are available in many public libraries.

An asterisk designates an out-of-print book which is useful or not replaced by a revision. Such books may be found in many libraries and sometimes on the secondhand market.

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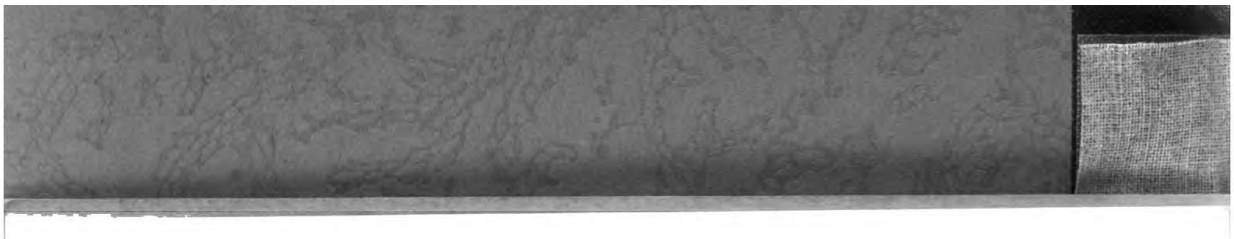

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